Legacies of Amazonian Dark Earths on Forest Composition, Structure and Dynamics

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To Demetrio and Gloria

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Chapter 1

General Introduction

Estela Quintero-Vallejo

The Amazonian rain forest is one of the most diverse biomes on the planet (Hoorn and Wesselingh 2010); its high biodiversity, although not completely understood, is the result of complex interactions between geomorphologic and climatic processes throughout thousands of years (Hoorn et al. 2010). It is this high diversity and complexity that fascinates people, and attracts worldwide efforts to keep this forest intact as a 'pristine', untouched ecosystem (Clement and Junqueira 2010). The claim of conserving an 'intact' forest is made under the assumption that the diversity of the Amazonian forest is mainly the result of natural processes (Peres et al. 2010). But the Amazonian forest does not seems not pristine, in the literal sense of the word, as it has gone through a long, and perhaps prevalent history of human occupation and modification (Heckenberger et al. 2007, Arroyo-Kalin 2012, Carson et al. 2014). Extensive presence of humans in the Amazonian region suggests an important contribution of mankind in shaping the Amazonian forest as it currently is (Balée and Erickson 2006, Heckenberger et al. 2007).

Past human inhabitation in the Amazonian forest is confirmed by the presence of large earth works in the South-West (Erickson 2006, Lombardo and Prümers 2010); and documented agricultural practices and use of fire for opening forested areas (Bush et al. 2007, Arroyo-Kalin 2012). Additional indication of human inhabitation in the Amazonian forest is the presence of anthropogenic soils or Amazonian Dark Earths- ADE ('*Terra Preta de Índio*' in Portuguese), named after the black, profoundly modified soil profile with increased organic matter (Sombroek 1966, Lehmann et al. 2003a). These anthropogenic soils are, presumably, formed between approximately 2400 yr BP and around 500 to 300 yr BP (Neves et al. 2003) by the accumulation of kitchen middens over long periods of time (Schmidt et al. 2014). They are widely distributed across the region (Winklerprins and Aldrich 2010, McMichael et al. 2014), and they increase the soil fertility at small scales (i.e. < 2ha) (Glaser and Birk 2012). This thesis evaluates the effects of ADE soil fertility on the composition, structure and dynamics of a Bolivian forest that is located in the Southern boundary of the Amazon basin. Assessing if ADE have lasting effects on the re-grown forest after abandonment of indigenous settlements improves our ability to determine and understand the drivers of species composition, forest structure and dynamics in the Amazonian forest.

In the following sections I will first introduce several historical aspects of human occupation of the Amazonian region with a focus on aspects that have resulted in ADE, especially in the case of North-East Bolivia (Llanos de Moxos) (Fig. 1.1); the aim is to contextualize my research in the historical ecology of the region. Secondly, I will highlight major aspects of soils and vegetation in the Amazonian forest with some emphasis on the findings of earlier studies that relate ADE with vegetation in Central Amazonian forest. Finally, I will present the research objectives, with a brief introduction to the study site and the thesis outline.

The human footprint in the Amazonian Forest

The romantic view of the Amazonian forest as the archetype of 'virgin' forests, untouched by humans, is far from reality (Clement and Junqueira 2010). Recent findings show increasing evidence of landscape modification of tropical forests by humans around the world (Willis et al. 2004) . Human influence has been pervasive through the whole American continent since around 12,000 years ago (McCann 1999); for instance, signals of inhabitation in the northern Bolivian Amazonia Bolivian Amazonia are as old as 10.000 yr BP (Lombardo et al. 2013); earth works consisting of ring ditches in the province of Beni in northeast of Bolivia date ~2300 yr BP (Erickson 2010); the presence of roads, moats and bridges at upper Xingu in Mato Grosso Brazil seem to have been built between 700 and 300 yr BP (Heckenberger et al. 2003); and ADE in Central Amazonian forest have been established since ~2400 yr BP (Sombroek 1966, Smith 1980, Lehmann et al. 2003b). Besides, signs of human use of fire (slash and burn) for incipient cultivations date from around 6000 yr BP in the Americas (Piperno 2009); the practice of slash-and-burn agriculture in the Amazon basin seems to be associated with cultivation of maize in the Western Amazonian forest (around 5800 yr BP) and cassava in the North-West (around 5000 yr BP) (Arroyo-Kalin 2012). Thus, the Amazonian forest by the time of the encounters with Europeans is a mosaic of natural and recovered landscapes after periods of human transformation along pre-Columbian times.

Figure 1.1 Map of Bolivia showing the location of Llanos de Moxos in the Northern region of the country and the location of La Chonta, the study site of this thesis.

After contact with Europeans in the early 16th century, the indigenous population was decimated due to diseases from the old world and slavery (Myers et al. 2003). Therefore, settlements were abandoned allowing forest regeneration. Besides, during the 17th and 18th centuries the remaining indigenous populations went through a process of transformation in their demographic patterns and land uses practices (Denevan 1992, Cleary 2001). For example, the decrease in population size, following indigenous depopulation, changed labour intensities for provisions (Walker 2000). The introduction of metal tools (i.e. metal axes, '*machetes*') changed the way how forests were cleared up for agriculture (Denevan 1992); and the introduction of animals such as cows and horses became important in the daily life of the missions in Moxos (Walker 2000). Consequently, the Amazonian forest has recovered after abandonment by indigenous people after their contact with Europeans, but has gone through more intensive landscape transformations by practices taught by the missionaries and colonizers.

Amazonian Dark Earths

General characteristics

Amazonian Dark Earth or '*Terra Preta*' (black earth in Portuguese) is a type of anthrosols (man-modified soils) characterized by a thick dark or gray top layer with presence of ceramic pieces and small fragments of charcoal indicating a direct relation with past indigenous inhabitation (Sombroek 1966, Smith 1980, Woods and Glaser 2004). It is still under debate whether these soils were intentionally created or not, and scientific evidence is accumulating for support of both possibilities in ADE sites. Non-intentional origin of ADE is associated with the accumulation of debris from daily life activities (WinklerPrins 2014). Intentional origin of ADE is associated with the piling up of kitchen remains and charcoal around the villages over hundreds of years; a practice that is also observed in current indigenous communities in the Xingu Region in Mato Grosso state in Brazil (Schaan et al. 2009). But regardless of its origin, what is relevant to the context of ADE is the fact that these soils are the result of sedentary patterns of inhabitation of the Amazonian forest (Arroyo-Kalin 2010, WinklerPrins 2014). Evidence indicates that pre-Columbian indigenous people inhabiting ADE areas were fisherman, hunters, fruits and nuts gatherers, and growers of manioc (*Cassava sativa*), maize (*Zea mays*), some tubers, and trees (Myers et al. 2003, Neves et al. 2003, Bozarth et al. 2009, Rebellato et al. 2009). This implies a semi-permanent or permanent cultivation that suggests human-induced openings and disturbances of the Amazonian forests.

The sizes of ADE patches range from less than 2 ha to more than 100 ha throughout the Amazon basin (Winklerprins and Aldrich 2010). Recent estimates of the presence of ADE in the region account for around 3.2% (McMichael et al. 2014), being distributed in Bolivia, Brazil, Ecuador, Guyana, Peru, and Venezuela (WinklerPrins 2014). The fertility of these soils is high when they are compared with the surrounding Ferralsols (Oxisols) and Acrisols (Ultisols), typical of the Amazon basin. In general, ADE soils contain high concentrations of total and available P (average of available phosphorus –P- higher than 35 ppm) and calcium (Ca) (average 7.1 cmol kg^{-1}), and elevated soil pH (values superior to 5.5) (Lehmann et al. 2003b). The increase in these elements is a consequence of addition and accumulation in the soil of animal bones (Lima et al. 2002), while the increase in soil pH can be a consequence of additions of cations (Ca, magnesium -Mg) to the soil (Costa et al. 2009). Nevertheless, there is a large variability in nutrient values among different ADE sites (Falcão et al. 2009). The black colour of these soils is mainly caused by high concentrations of organic matter, charcoal and carbon (C) (Glaser et al. 2003). It is believed that it is the high charcoal concentration in the soil that contributes to nutrient retention over long periods of time (Glaser et al. 2003, Lehmann et al. 2003b, Glaser 2007, Novotny et al. 2009). This explains that, even though the age of these soils dates from ca. 2400 to 500 yr BP (Neves et al. 2003), the nutrient levels seem to be stable over time.

Currently, ADE are used for cultivation of home garden, agroforestry, and slash-and-burn agriculture (Hiraoka et al. 2003, Clement et al. 2009, Junqueira et al. 2010, Junqueira et al. 2011). People cultivate in these soils because crop biomass is higher, e.g., the production of rice and beans doubled when cultivated on ADE compared with adjacent ferralsos (Oxisols) (Lehmann et al. 2003b). Additionally, the time of the cropping period can be reduced until four times on ADE respect to surrounding non-ADE soils (German 2003, Major et al. 2005a, Major et al. 2005b). In some sites, communities in the Amazonian region gather fruits and wood from ADE sites that undergo a fallow period and are occupied by secondary forest (Clement et al. 2009, Junqueira et al. 2010).

Amazonian Dark Earths and ancient human forest use in Bolivia

Past human history of the Bolivian Amazonian forest has been documented mainly in the Llanos de Moxos in Beni department, Bolivia, where monumental earthworks composed of rings, causeways, and dikes are the landmark of the landscape (Erickson 2006, 2008, Lombardo and Prümers 2010). Recent studies have also documented the presence of ADE in the area with presence of ceramics, high soil fertility (Walker 2011) and elevated values of soil pH and P (Hastik et al. 2013). About 250 Km to the South-East of Llanos de Moxos (Fig. 1.1); there is evidence of ADE presence in a forested area ceramics and high levels of soil pH, P and Ca (Calla 2003, Paz-Rivera and Putz 2009).

The human historical context of the region of Llanos de Moxos is associated with populations of gatherers since ~10.000 yr BP (Lombardo et al. 2013), fisherman (355 yr BP), and farmers probably since ~1600 y BP (Whitney et al. 2014). Besides of the construction of raised fields (Erickson 2006), inhabitants of the region used fire possibly associated with agricultural practices (Whitney et al. 2014). These fire practices have been, however, dynamic through time decreasing around the middle of the 17th century (Whitney et al. 2014). Chronicles of the 16th century that relate the encounters between Spaniards and missionaries with local inhabitants, describe the regions of Llanos the Moxos inhabited by a wide variety of small communities, apparently each with its own language and settlements conditioned to the geographical context. Thus, there where communities with 'amphibian' cultures used to regular flooding, and practicing agriculture in elevated zones; other communities in the region of Baures opened small areas with fire to cultivate products such as bitter and sweet manioc, peanuts, squash and peppers. Additionally, fishing when communities were living close to rivers, and hunting of peccaries, deer, tapir, are described as the main ways of living (Block 1994).

The human history before the contact with Europeans of the region outside from the Llanos de Moxos (where this study took place) is hardly documented. It seems that by the end of the 17th century, Jesuits missionaries explored the region, describing the presence of around 60 different towns of around 60 people each (Van der Berg 2006). The inhabitants of this region, which are called Guarayos, were closely related to the Tupi-Guaraní speaking indigenous people that migrated from the south, today Paraguay, around the 13th century (Calla 2003, Paz 2003). The Guarayos lived in small groups in the forest, and hunted and practiced agriculture (Calla 2003). With the influence of missionaries, they started to use metal tools that allowed them to open the forest, creating gaps for cultivation of useful plants, and to establish home gardens (Van der Berg 2006). Explorers at the start of the 19th century describe the practices of the Guarayos as a shifting cultivation system with crops of corn, cassava, and squash (D'Orbigny 1847).

Soil and vegetation in the Amazonian forest

Soils and species composition

Forests in the Amazonian region are shaped mainly by a complex geomorphologic history that influences soil nutrients and climate that determines flooding and drought seasonality (Hoorn and Wesselingh 2010, Stropp 2011). At large scale, tree diversity patterns and tree species composition can be explained by current climate and soil fertility levels. Broad climate patterns range from seasonal sites (> 1 month with < 100mm of rain) in the South-East to non-seasonal sites (0 to 1 month with <100mm of rain) in the North-West (ter Steege 2010, Stropp 2011). Soil fertility ranges from poor soils that originated from the Precambrian (Guyanese and Brazilian) shields in the Eastern region to richer soils that originated from sediments during the origin of the Andes in the Western region (Quesada et al. 2010, Quesada et al. 2011). Thus tree α-diversity is lower in the North-East and increases towards the west, with Eastern forests being composed by a higher proportion of Leguminosae, high wood density, and larger seeded species (ter Steege et al. 2006, ter Steege 2009, 2010, Quesada et al. 2012). Other plant groups show similar distributional patterns; for example, fern species diversity increases with a gradient of cation concentration in the soil that goes from north-east to south-west (Tuomisto et al. 2014). Similarly, changes in fern composition follow changes in cation concentration in the soil observed in the Amazon basin (Zuquim et al. 2014).

At local scale (mainly < 1 km^2) diversity is constrained by dispersal limitation and environmental filters (ter Steege 2010). When soils are studied at a small scale (50 ha), the ability to correlate plant distribution with soil properties is improved (John et al. 2007, Dalling et al. 2012). In the case of the Lambir 50 ha plot in the peninsula of Malaysia, small-scale variation in soil chemistry was correlated with tree distribution; in particular, Mg and P drove these patterns (Davies et al. 2005). In tropical regions such as forest of Panama, Ecuador and Colombia, distribution of more than one third of the studied tree species was associated with soil nutrients (John et al. 2007). Distribution of tree species in the Amazonian forest responded to soil resources and hydrological conditions related to topography (Duque et al. 2002, Schietti et al. 2014). Similar associations with soils have been found for other plant groups. In western and central Amazonian forest, floristic composition of understory ferns, herbs and palms was correlated with clay percentage and cation concentration of Ca, Mg, K and Na (Tuomisto et al. 1998, Costa et al. 2005, Poulsen et al. 2006, Jones et al. 2008, Zuquim 2008). Besides, fern and Marantaceae diversity increased with cation concentration in the soil in central and western Amazonian forest (Tuomisto et al. 1998, Costa et al. 2005, Costa 2006).

Amazonian Dark Earth and species composition

Some studies have evaluated the effect of ADE on plant distribution in the central Amazonian forests. In abandoned crop lands close to the city of Manaus, both diversity and biomass of weeds were high on ADE when compared to abandoned crop lands on adjacent non-ADE soils (Major et al. 2005b). In another study carried out on secondary forest of until 30 years after abandonment, plots established on ADE and on adjacent soils (non-ADE) shared only 46% of understory palm species and only 23% of the woody species (Diameter at Brast Height [DBH]>5cm) (Junqueira et al. 2010). A study comparing an old growth forest (between 300 and 700 years old) on ADE with forest of non-ADE found that only ~7% of species (>10cm stem diameter) were shared between these forests, and that tree diversity was lower on ADE soils (Clement et al. 2009).These results suggest that ADE affects the distribution of some plant species, mainly due to cultivation of some useful species in sites of ADE or close to it (Junqueira et al. 2010, Junqueira et al. 2011). Nevertheless, results from an old growth forest (~ 480 yr BP) in the southern border of the Bolivian Amazonia showed that density of man-used tree species was similar between ADE sites and non-ADE sites (Paz-Rivera and Putz 2009).

In this thesis I evaluate the effect of ADE on understory herb, fern, and palm species composition and diversity (Fig. 1.2). Given that nutrient levels of ADE are high, fertility of the soils could have an effect on angiosperm herb, fern and palm species composition and richness (Costa et al. 2005, Major et al. 2005b, Costa 2006, Poulsen et al. 2006, Zuquim et al. 2012) (Fig. 1.2). Additional to soil effects, composition, richness and abundance of palms can be the consequence of the use of these species by humans (Levis et al. 2012).

Soils and forest structure and dynamics

Soil properties do not only affect species distribution and composition, but also drive forest structure. In the whole Amazonian forest basal area and liana density increase with the amount of soil nutrients (Malhi et al. 2004, Aragão et al. 2009, Quesada et al. 2009), while in a regional study in the Bolivian Amazonia the density of trees and palms and the liana infestation show the opposite trend (Toledo et al. 2011a). At local scale, aboveground biomass increased with increasing soil nitrogen (N), and percentage of clay (Laurance et al. 1999), and differences in texture caused by topography such as higher elevations (de Castilho et al. 2006). Besides, trees > 30 cm DBH had higher aboveground biomass in more fertile soils compared with less fertile soils in western Amazonian forest (DeWalt and Chave 2004).

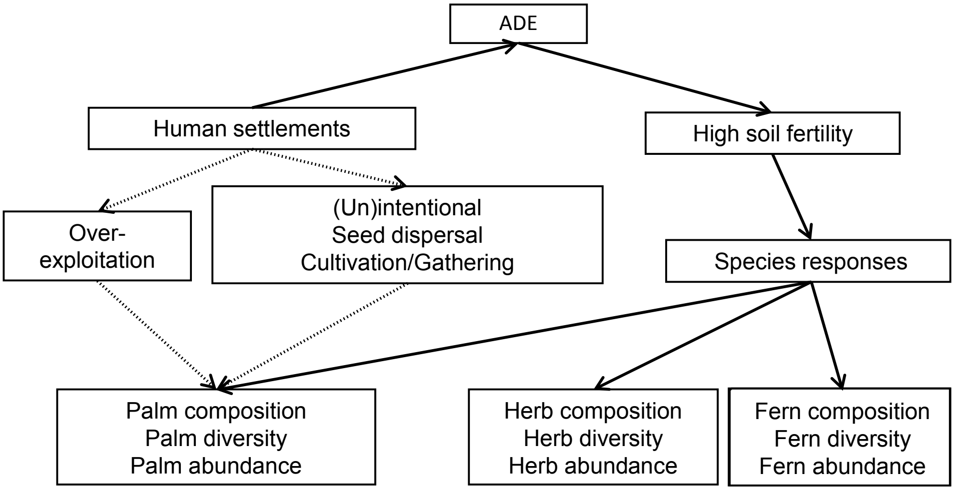

Figure 1.2 Conceptual diagram of the relationships between ADE soils and species composition, diversity and abundance. The increase in soil fertility on ADE could determine species composition through species responses to soil fertility. Similarly, soil fertility will affect herb and fern diversity and abundances. These relationships are indicated with solid arrows and will be evaluated in this thesis. Species that historically had been used by humans, such as palms, can show responses to soil nutrients but their composition, diversity and abundance can also be affected by management of past inhabitants of the area of La Chonta. These relationships are indicated with dashed lines, and will not be directly evaluated in this thesis.

In addition to the effects of soil in forest structure, forest dynamics have been shown to be strongly associated with soil characteristics. During the past 25 years, tree recruitment and mortality rates have increased in the whole Amazon basin, with recruitment rates being higher than mortality rates (Phillips et al. 2004). This increase was higher in the fertile western than in the more infertile eastern Amazonian forest (Phillips et al. 2004). The mechanisms that explain this pattern are not fully understood yet, but the increased dynamics can be associated with changes in species composition along a gradient of soil fertility. On one hand, sites with higher growth and mortality rates are associated to species with low wood density (Poorter et al. 2008), perhaps because of the presence of more pioneer or long-lived pioneer species in the west. On the other hand, sites with lower mortality and growth rates are associated high wood density, in this case more shade tolerant species in the east (ter Steege et al. 2006, Quesada et al. 2012).

Forest productivity can be also affected by soils. Total net primary productivity (NPP) was related to soil fertility across 10 1-ha plots distributed across the Amazonian forest. These sites covered a wide spatial gradient of soil fertility with plots located on sites with low nutrient soils such as white sands and on sites with high nutrients such as an ADE site. Thus, on these contrasting soils, NPP increased almost twice in the soils with high nutrient availability specially available P, compared with the less fertile soil (Aragão et al. 2009). Similarly, a forest growing on ADE had higher gross primary productivity (GPP), NPP, and high rates of fine root production compared with a forest growing on non-ADE (Doughty et al. 2014).

In this thesis, I evaluate the effect of nutrients increases in ADE on forest structure and dynamics. Increases in soil P, for example, directly could affect basal area (Quesada et al. 2009), tree growth (Aragão et al. 2009) and lianas (DeWalt et al. 2006). Increases in growth and mortality rates (Quesada et al. 2012) could create gaps in the forest that could influence the presence of lianas, which in turn, could negatively affect tree mortality (Phillips et al. 2005). Likely, an increase in nutrients in the soil would shift species composition to more fast growing species, which will have an effect on the forest dynamics (Quesada et al. 2012) (Fig. 1.3).

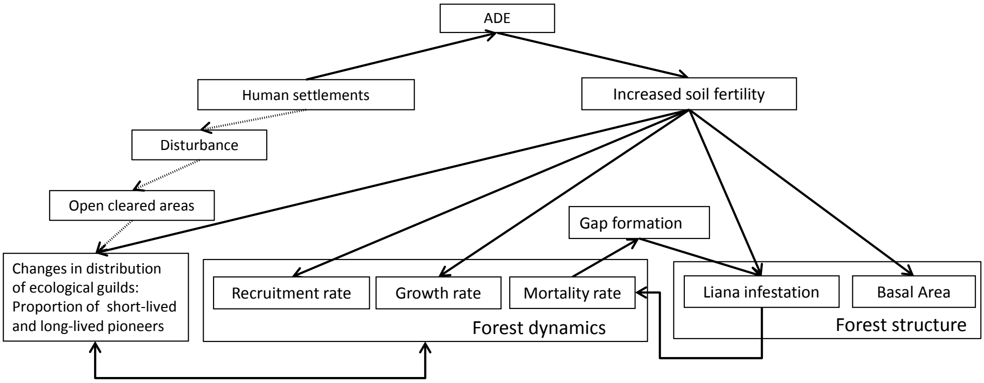

Figure 1.3 Conceptual diagram of the relationships between ADE soils and forest structure and dynamics. Increased soil fertility can affect forest structure (by increasing basal area and liana infestation) and forest dynamics (by increase tree growth). Increases in tree growth could affect mortality rates which affect gap formation and modify forest structure. Increases in nutrients on ADE could affect species composition favouring fast-growth species that affect forest dynamics. These relationships are evaluated in this thesis and are represented by continuous arrows. Disturbance generated by human inhabitation also could influences species composition; this association is shown by dashed lines and will not be directly evaluated in this thesis.

Plant responses to soil nutrients

Plant responses to nutrients are intended to maximize growth rates (Poorter and Nagel 2000) and minimize mortality (Walters and Reich 2000). Although there is no unique strategy for growth maximization, in poor nutrients environments plants generally respond in a conservative way by storing carbon and nutrients in denser and tougher tissues or increasing organ life span (Reich et al. 1991, Eissenstat et al. 1997, Hill et al. 2006). In fertile environments, plants generally respond in an acquisitive way by increasing specific leaf area (SLA) and nitrogen (N) and phosphorus (P) concentration in leaves (Ordoñez et al. 2010). But these responses can be modulated by ways in which plants use and incorporate nutrients from the soil leading to a specific elemental composition (Sardans and Peñuelas 2014). For example, the increase in soil fertility will increase the growth of saplings of tropical trees except for legume species (Baribault et al. 2012). Among nutrients, N and P are the major limiting elements for primary productivity of forests (Vitousek et al. 2010). Nevertheless, recent studies have found that potassium (K) and Ca influence plant growth in tropical forest in similar ways as N and P (Wright et al. 2011, Baribault et al. 2012).

In this thesis I evaluate the responses of tree seedlings to nutrients in ADE using a controlled experiment. Assessing changes in plant traits associated to fertility of ADE provides a unique opportunity to understand tree responses to soil fertility, especially responses to specific nutrients such as P and Ca. These two elements are gaining importance in explaining the CO_2 uptake of tropical trees (Asner et al. 2014). Previous studies on crops have shown that in fertile environments such as ADE, rice and beans have doubled the production compared with crops cultivated on natural Ferralsols in the Amazonian region (Lehmann et al. 2003b). For this reason, ADE are often used by local farmers to grow crops faster and for longer periods of time compared with slash and burn agriculture done on regular soils in the Amazon basin (German 2003).

Research objectives and hypotheses

This research aims to evaluate the effect of Amazonian Dark Earths soil fertility on species composition and forest structure and dynamics in a southern Amazonian forest in Bolivia, and to understand possible mechanisms that explain patterns of plant distribution and forest dynamics at local scales.

Specifically, this study evaluates:

1) The effects of ADE on the composition, diversity and density of plant life forms that respond to soil nutrients such as angiosperm herbs, ferns and palms.

2) The effects of ADE on forest structure and dynamics; evaluated through empirical field data;

3) The effects of ADE on the nutrients through the evaluation of growth and performance of tropical tree seedlings in a controlled experiment. With this experiment it will be possible to unravel the mechanism that explain species responses to ADE.

Hypotheses

Human-induced changes through ADE might change characteristics mainly related to soil fertility. For example, charcoal addition potentially leads to more stable organic matter, higher nutrient levels and probably to higher soil water retention capacity in ADE compared to non-ADE soils (Glaser et al. 2002), while the addition of organic waste leads to higher nutrient (P, N, and Ca) concentrations (Glaser 2007). Higher Ca concentrations increase the base exchangeable concentration and pH of the soil, which in turn also increases the availability of other cations (Lambers et al. 2008a). The increase in fertility of ADE could affect forest composition through species-specific responses to soil nutrients and plant growth that ultimately determines forest structure and forest dynamics (Fig. 1.4). Finally, forest species composition can be also affected by human enrichment of useful plants in or close by inhabitation (Fig. 1.4), but this is probably not the case in La Chonta forest because previous studies did not found strong associations between species useful for humans and past settlements (Paz-Rivera and Putz 2009).

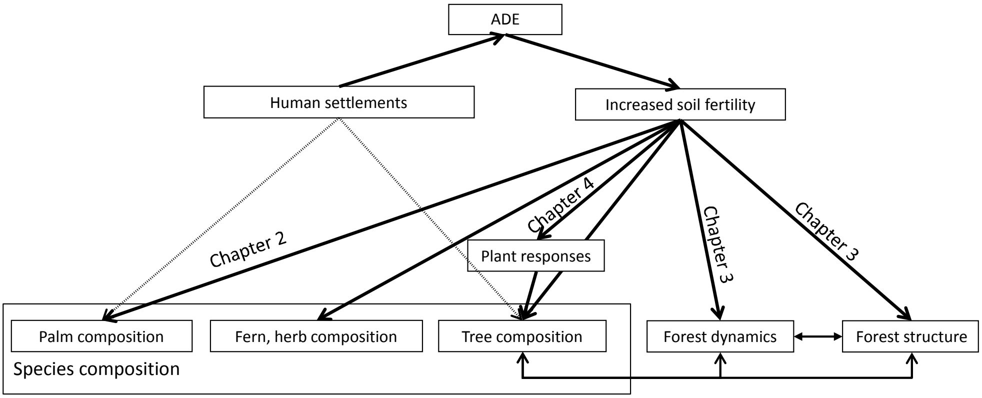

Figure 1.4 Conceptual framework of the research reported in this thesis concerning the effect of Amazonian Dark Earths (ADE) on vegetation attributes. Soil and vegetation are connected, in this thesis, through changes in forest composition (Chapter 2), structure and forest dynamics (Chapter 3), that can be a consequence of specific relationships between the increase in fertility of ADE and plant responses (Chapter 4). Continuous arrows represent the relationships that are directly evaluated in this thesis. Dashed arrows represent weak effect of humans on species composition for La Chonta forest through selective harvesting and enrichment activities (Paz-Rivera and Putz 2009), which are not directly evaluated in this thesis.

Thesis outline

This dissertation consists of five chapters including this general introduction (chapter 1), three research chapters (chapters 2-4) and a chapter that synthesizes the results (chapter 5). To identify sites where ADE were present, I screened the soils in 12, 27-ha permanent plots of La Chonta forest by characterizing soil colour and recording the presence of ceramics. I established small plots to relate understory species composition (chapter 2) and forest structural and dynamic variables (chapter 3) to ADE soils characteristics. Besides, I used a greenhouse experiment to test whether ADE soil collected at La Chonta affected tree seedlings performance (chapter 4).

In the Amazonian region, soil nutrients can play a role in the distribution of understory species (Costa 2006, Zuquim et al. 2014). In chapter 2, I evaluated the effect of ADE changes in soil nutrients on understory species composition of an Amazonia Bolivian forest. I screened soils by looking for sites where ADE could be present and I recorded the composition of terrestrial ferns, angiosperm herbs and understory palms. These groups of plants were selected because it has been shown that they respond to nutrients in the soil (Vormisto et al. 2004, Costa 2006, Costa et al. 2009, Zuquim et al. 2014). Additionally, by looking at understory species such as ferns I could separate the effect of soil alone from the effect of human enrichment as, to this date, ferns have not been reported as being used by humans in the past, which, in the contrary, is the case for palms and herbs. Subsequently, I correlated soils variables associated with ADE with species composition, diversity and abundance of these three groups of plants.

Soil fertility has an effect on forest structure and dynamics throughout the Amazonian forest. Nevertheless, at small scales the role of soils in forest dynamics in the Amazonian forest is less clear (Phillips et al. 2004, Malhi et al. 2006). Studying forest structure and dynamics at local scale will improve our ability to separate the effects of edaphic variables from topographic variables and from successional sequences (Pan et al. 2013). Knowing the drivers of forest growth will contribute to improve forest production for C sequestration and climate change mitigation (Malhi et al. 2009). In chapter 3, I tested the hypothesis that increased fertility of ADE soils could have an effect on forest structure and forest dynamics as a result of its effects on tree growth, tree recruitment and tree mortality. I used long term (~10 year) data on forest dynamics of 12 large permanent plots (~27 ha each), and a statistical modelling approach to relate soil variables (pH, P, N) with forest attributes (stand basal area, liana infestation, and successional composition) and dynamics (recruitment, mortality and growth). The effect of soils was evaluated in two steps: first I determined the effect of soils on forest attributes and then I determined the effect of soils and forest attributes on forest dynamics.

Increases in soil nutrients can drive plant responses such as increases in growth and decreases in mortality, which can lead to successful establishment and species regeneration in the forest (Poorter and Nagel 2000, Walters and Reich 2000, Fine and Kembel 2011). In chapter 4, I tested the hypothesis that the increase in nutrients in ADE increases seedling growth of selected tree species, and that this increase is a consequence of changes in plant traits. To address this question I conducted a shade-house experiment with seedlings of 17 tree species. I planted them in ADE and non-ADE collected in La Chonta forest and grew them for ~4 months.

Finally, in chapter 5 I discuss how ADE can be considered a human legacy in this southern Amazonian forest, that affects species composition and forest structure. First, I contextualize the ADE characteristics in the southern Amazonian region (where background soil fertility is high) and discuss the main differences with ADE from the central Amazonian forest (where the background soil fertility is much lower). Second, I discuss the effects of nutrient addition on a fertile soil on individual plants and forest growth. Lastly, I discuss the human legacies in the soil and in the vegetation and the indirect human legacies in the vegetation through the soil.

Study site

This study was carried out in the forestry concession of Agroindustria Comercial La Chonta, on Guarayos province, Santa Cruz Department, Bolivia (15º47'S, 62º55'W). This is a 100,000 ha semi-deciduous tropical moist forest. Mean annual temperature is 24.3 °C and annual rainfall is 1580 mm (data from 2000-2006 from La Chonta sawmill) with a dry season (<100 mm/month) from May through September (Peña-Claros et al. 2012).

La Chonta is located in a confluence between old and young soils. The old soils are an extension of the Precambrian Brazilian Shield, whose southern boundary is around 250 km from the concession, and the young soils overlaid the basement rock with alluvial deposits from the Tertiary and Quaternary (Burbridge et al. 2004, Gosling et al. 2005) that are a consequence of the uplifting of the central Andes (Latrubesse et al. 2010). Soils at La Chonta can be mostly Cambisols, although there are also Histosols, Gleysols, Leptosols/Regosols and Anthrosols (see Appendix of this thesis). Cambisols are generally young, and not necessarily very fertile. Histosols and Gleysols are soils associated with lower areas in the landscape. Leptosols are thin soils, not very deep, with coarse material, and presence of gravel very close to the soil surface; they are found in the top of the small hills. Finally, Anthrosols are present in La Chonta, indicated by the presence of a horizon with dark soil color (Munsell 7.5YR 2.5/1) and remains of artefacts, such as pieces of ceramics or human generated charcoal.

The forest at La Chonta can be classified as a semi-deciduous tropical moist forest (Peña-Claros et al. 2012). Floristic composition suggests that the forest at La Chonta is transitional between the forest of the northern Amazonian region and the southern Chiquitano dry forest, with a higher abundance and frequency of *Pseudolmedia laevis*, *Licaria triandria* and *Ampelocera ruizi* (Toledo et al. 2011a). This mixture of dry and wet forest species seems to be related with the paleo-climatic history of the region that was a mixture of seasonal forest and savanna between 50,000 and 11,400 yr BP and only had non-seasonal species and species of humid and wet forest appearing in the paleo-palynological record between 6,000 and 2,000 yr BP (Mayle and Whitney 2012). The forest has an average height of 27 m, and approximately one third of the canopy species shed the leaves in the dry season (Markesteijn et al. 2010). The forest has, on average, 367 stems (>10 cm DBH) and 59 species per ha (Peña-Claros et al. 2012).

The forestry concession of La Chonta was established in 1974. During its first 20 years, the concession logged only mahogany trees (*Swietenia macrophylla*) and tropical cedar (*Cedrela fissilis*). Before this time there was no evidence of forest management in the area.

Nevertheless, the presence of ADE soil with pieces of charcoal and pottery of 400 to 300 yr BP indicates that there were indigenous settlements in the area (Paz 2003). Currently, the concession harvests around 3 trees/ha (7.7 m^3/ha) belonging to 17-19 tree species. Besides, in this area the Bolivian Forest Research Institute (IBIF, in Spanish: Instituto Boliviano de Investigacion Forestal) carries out the Long-term Silvicultural Research Program (LTSRP) that consist in 12 large-scale (27 ha) permanent sample plots distributed in three logging compartments, which were harvested by La Chonta company between 2001 and 2002, after plot establishment (Peña-Claros et al. 2008).

The forest of La Chonta is an interesting forest to study ADE and test hypotheses about the effects of ADE on forest composition, structure and dynamics because of the following reasons: First, given its borderline location in the Amazon River basin, La Chonta is one of the most southern sites with ADE reported until present; Secondly, In La Chonta ADE is located in sites that are around 14 to 30 km distant from the rivers Blanco and Negro, respectively (Paz-Rivera and Putz 2009), which is not common for ADE sites; Thirdly, differently from most of the reported ADE sites in the Amazonian region, ADE underlying soil is fertile; Finally, the Instituto Boliviano de Investigacion Forestal (IBIF, in Spanish) has established permanent plots since 2000 making regularly censuses that brings a unique opportunity to evaluate the effects of increases in soil fertility on forest dynamics and structure.

Institutional context

This study was conducted within the framework of the *Terra Preta* Program (http://www.terrapretaprogram.org/). The program was initiated in 2010, with funds of INREF Interdisciplinary Research and Education Fund (The Netherlands), and associated partners from Brazil (EMBRAPA, Instituto Nacional de Pesquisas da Amazonia), Bolivia (IBIF, Instituto Boliviano de Investigacion Forestal), and Colombia (Universidad Nacional de Colombia).

This research programme integrates social and natural sciences in order to understand the context in which the *Terra Preta* phenomena originated, the current uses and practices on T*erra Preta* by Amazonian inhabitants; and its future as possible alternative for a sustainable agriculture in the Amazonian region (Fig. 1.5). For these purposes, the program consist of 6 Ph.D. interdisciplinary projects aiming to integrate information on the opportunities and disadvantages of ADE for their sustainable use and management. The contents of expected dissertations are briefly outlined below:

1. The effect of Amazonian Dark Earths on the composition, structure and dynamics of a Bolivian forest (this thesis)

2. The current use of anthropogenic soils by local people in Central Amazonia (André Braga Junqueira)

3. People, soils and manioc interactions in the upper Amazon region (Clara Patricia Venegas)

4. The recreation of *Terra Preta* 'nova' by understanding the effects of fire in processes of char production in Brazil (Paulo Paiva).

5. Challenging the claims on the potential of biochar to mitigate climate change (Tatiana Rittl)

6. The role of black carbon (BC) and its interactions with soil Ca, and P in the stabilization of soil organic matter (SOM) in Brazil (Carlos Alho).

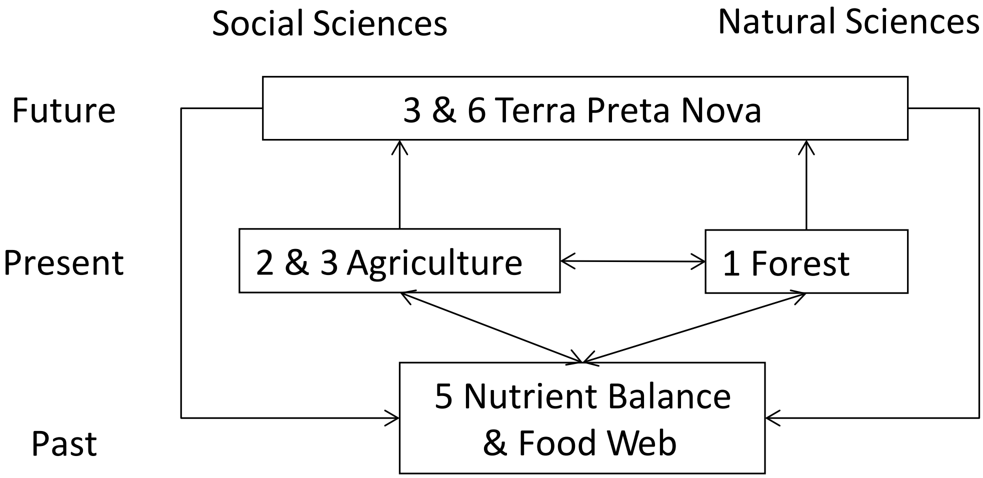

Figure 1.5 Diagram showing how the social and natural sciences are combined in the *Terra Preta* Program. Note that the different studies allow connecting different time scales: past, present and future. Numbers refer to the six PhD research topics included in the program. Research topic 1 was carried out in Bolivia, research topic 3 in Colombia, and research topic 2, 4, 5 and 6 in Brazil.

Chapter 2

Amazonian Dark Earths Shape the Understory Plant Community in a Bolivian Forest

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Abstract

Amazonian Dark Earths (ADE) are the result of human modification of the Amazonian landscape since pre-Columbian times. ADE are characterized by increased soil fertility compared to natural soils. In the Amazonian forest, soil fertility influences understory herb and fern species composition. However, little research has been done to evaluate the effect of ADE on the composition of the understory community. We evaluated the effects of ADE and soil in 36 plots (150x4m) established in a Bolivian moist forest (La Chonta). For each plot we determined soil nutrients, and the composition, richness, and abundance of terrestrial fern, angiosperm herb, and understory palm species. We found that the presence of ADE created a gradient in soil nutrients and pH that affected the understory species composition, especially of ferns and palms. Additionally, the higher nutrient concentration and more neutral soil pH on ADE soils caused a decrease of fern species richness. We therefore conclude that the current composition of the understory community in this particular Bolivian forest is a reflection of past human modifications of the soil.

Keywords: Anthrosols; terrestrial angiosperm herbs; terrestrial ferns; soil pH; understory palms; Bolivia; T*erra Preta*.

Introduction

Plant diversity and composition are driven by historical events, dispersal limitation, and biological (Fine et al. 2004), environmental (ter Steege 2009), and anthropogenic filters (Ross 2011). These filters determine the locations that are adequate for germination, growth and survival of plants (Fine and Kembel 2011). In Amazonian forest, edaphic characteristics are one of the most important environmental filters, shaping the floristic composition and diversity of understory communities. Several studies have shown that exchangeable base concentration and to some extent clay percentage in the soil influenced the distribution patterns of understory fern, angiosperm herbaceous and palm species (Costa et al. 2005, Poulsen et al. 2006, Costa et al. 2009, Kristiansen et al. 2012, Zuquim et al. 2014). Also it has been found that exchangeable base concentration positively affected species richness of Marantaceae and ferns (Costa 2006, Tuomisto et al. 2014) while it negatively affected palm species richness in western Amazon basin (Vormisto et al. 2004).

Plant distribution is not only shaped by biological and environmental factors, but also by anthropogenic factors. Humans may change the environmental conditions (e.g., light, soil nutrients), and hence plant distribution through disturbance of the vegetation (e.g., shifting cultivation) and soil modification. Humans also may directly impact species distribution by harvesting useful species or by deliberately planting them. In Central Amazon, for example, there is a higher abundance of useful tree and palm species in areas surrounding past human settlements (Levis et al. 2012). Pre-Columbian civilizations have strongly affected the environment in several ways: through the presence of roads, moats, and bridges at upper Xingu in Mato Grosso Brazil (Heckenberger et al. 2003); earth works in the province of Beni, Bolivia (Erickson 2010); and the pervasive existence of Amazonian Dark Earths (ADE) in the Amazon basin (Sombroek 1966, Glaser and Birk 2012). In this paper, we focus on ADE or black earth ('*Terra Preta*' in Portuguese), which is a type of anthrosol characterized by a thick dark or gray top layer with presence of ceramics indicating past indigenous settlements (Sombroek 1966, Woods and Glaser 2004). ADE are the product of kitchen remains, such as garbage deposition, over thousands of years (Schaan et al. 2009). ADE patch sizes range between 2 ha to more than 100 ha (Winklerprins and Aldrich 2010), and possess higher concentrations of total and available phosphorus (P) and calcium (Ca) (from the addition of animal bones to the soil), higher total nitrogen (N) (Lehmann et al. 2003b), and higher concentrations of charcoal and carbon (C) (Glaser et al. 2003) than the surrounding ultisols and oxisols from most of the Amazonia (Falcão et al. 2009). When ADE soils are present in a given area, they form a gradient of fertility from the center of the ADE patch to the surrounding natural soils (Fraser et al. 2011, Costa et al. 2013).

Human-induced changes in soil structural, physical, and chemical properties, and soil processes have the potential to have long-lasting effect on the understory vegetation. For example, charcoal addition leads to more stable organic matter, nutrient levels and probably to higher soil water retention capacity in ADE compared to non-ADE soils (Glaser et al. 2002), while the addition of organic waste leads to higher nutrient concentrations (P, N, and

Ca) (Glaser 2007). Higher Ca concentrations increase the base exchangeable concentration and pH of the soil which in turn, also increases the availability of other cations. In combination, the increased nutrient storage and nutrient availability enhance plant nutrient uptake (Neumann and Römheld 2012). The interplay between nutrient supply and species-specific nutrient demands determine in the end where species occur. At the lower end of the soil fertility gradient an increase in nutrients has the potential to fuel plant growth, and enhance species abundance and richness. At the higher end of the soil fertility gradient species richness may even decline, when nutrient availability becomes supra-optimal, or when fast-growing species dominate and outcompete the others (Grime 1973).

So far, few studies have examined the possible effect of ADE and associated human activities on plant distribution and floristic composition. In recently abandoned croplands there is a higher diversity and biomass of light-demanding weeds on ADE soils than in adjacent non-ADE soils (Major et al. 2003, Major et al. 2005b). Young secondary forests on ADE and non-ADE soils shared only 23% of woody species (palms and trees with diameter to breast height [DBH] > 5cm) and 46% of understory palm species. This low similarity was attributed to differences in soil fertility and to the enrichment with useful tree and palm species at ADE sites (Junqueira et al. 2010, Junqueira et al. 2011). Finally, the only study performed in an old-growth forest showed that density of human-used tree species was similar between ADE and non-ADE sites in the Bolivian Amazonia (Paz-Rivera and Putz 2009).

In this paper, we evaluate the effect of ADE soils on the composition and diversity of understory angiosperm herbs (hereafter referred to as herbs), ferns, and palms in an Amazonian Bolivian forest. We tested the hypothesis that the physical and chemical characteristics of soils differ along a gradient from ADE to non-ADE soils. We then tested the hypothesis that floristic composition, species richness and abundance of understory herbs, ferns and palms differ along the gradient from ADE to non-ADE soils. We predict that soil fertility and soil organic matter (and with that indirectly also soil water content) would increase along the gradient from non-ADE to ADE soils; and that species richness and abundance of herbs, ferns and palms would increase with soil fertility and soil organic matter (and thus soil humidity), as has been reported elsewhere in mostly oligotrophic Amazonian forests (Tuomisto et al. 2002, Vormisto et al. 2004, Costa et al. 2005, Tuomisto et al. 2014).

Methods

Study site

The study was conducted in the 100,000 ha forestry concession of Agroindustria Comercial La Chonta, Guarayos province, Bolivia (15º47’S, 62º55’W). Mean annual temperature is 24.3 °C and annual rainfall is 1580 mm (data from 2000-2006 from La Chonta sawmill) with a dry season (< 100 mm/month) from May through September (Peña-Claros et al. 2012). The study site is located on the South-West border of the Brazilian Precambrian Shield (Navarro and Maldonado 2002) and has sandy-loam soils with neutral pH and rich in nutrients (Paz 2003, Peña-Claros et al. 2012). A previous study showed that ADE soils in La Chonta forest cover about 20 percent of a studied area of 216 ha (Paz-Rivera and Putz 2009). The forest at La Chonta can be classified as a semi-deciduous tropical moist forest (Peña-Claros et al. 2012). Floristic comparisons of several lowland forests in Bolivia show that the forest at La Chonta is transitional between the forest of the Northern Amazonia and the Southern Chiquitano dry forest (Toledo et al. 2011a). The forest canopy has an average height of 27 m, and approximately one third of the canopy species shed the leaves in the dry season (Markesteijn et al. 2010). The forestry concession of La Chonta was established in 1974. Currently, they harvest around 3 trees/ha (7.68 m^3/ha) belonging to 17-19 tree species. During its first 20 years, the concession logged only trees of mahogany (*Swietenia macrophylla*) and tropical cedar (*Cedrela odorata*). Before this time there was no evidence of forest management in the area. Nevertheless, the presence of ADE soil with pieces of charcoal and pottery of 400 to 300 yr B.P. indicates that there were indigenous settlements in the area (Paz 2003). Research was carried out in twelve large-scale (27 ha) permanent sample plots established by the Bolivian Forest Research Institute (IBIF, in Spanish), hereafter referred as the IBIF-permanent plots, that are part of the Long Term Silvicultural Research Program, LTSRP (Peña-Claros et al. 2008). Plots were distributed in three logging compartments, which were harvested by the company between 2001 and 2002, after plot establishment.

Data collection

First, we performed a preliminary survey in the IBIF-permanent plots, using the grid system of the 27 ha plots to make an inventory of the presence of ADE soils. At each 50 x 50 m grid intersection of each plot, a soil sample was cored with an auger, providing 130 sample points per plot, for a total of 1750 points. Presence of ceramics and charcoal was evaluated visually, and the soil colour was determined with Munsell Soil Color Charts. Subsequently, we made soil color maps using the Kriging interpolation function of ArcMap software (Klomberg 2012). Based on the soil color maps and the presence of ceramics and charcoal, we established a total of 36 rectangular plots (150 x 4 m). Fourteen plots were established in areas with dark soil (black or very dark brown color, mainly 7.5YR 2.5/1, 7.5YR 2.5/2 and 7.5YR 2.5/3 in the Munsell system); these plots were considered ADE plots. Fourteen additional paired plots were established on adjacent areas with lighter soil color (brown, yellowish brown or reddish brown, mainly 7.5YR 4/3, 7.5YR 4/4, 7.5YR 5/4, 5YR 5/3); these were considered as non-ADE soil plots. Distance between adjacent plots ranged between 120 and 400 m. Four additional pairs of plots on ADE sites previously determined by (Paz-Rivera and Putz 2009) were established outside the IBIF-permanent plots, so that the total sample size was 36 plots. We took geographical reference points of every transect using a GPS (Garmin *Etrex* Vista HCx, USA).

In each plot, we counted individuals belonging to fern species and species of the Marantaceae and Arecaceae families, as these have been shown to respond to soil fertility (Costa 2006, Poulsen et al. 2006, Andersen et al. 2010a). We also included other families that were easy to recognize and abundant in the understory of La Chonta, such as Commelinaceae, Costaceae, Heliconiaceae, Poaceae, and Zingiberaceae (Appendix2 Table A2.1). For species of small size, such as most fern species, we distinguished individuals separately. However, several species grow in clumps, therefore hampering the counting of individual plants. Stems of small clump-forming species (e.g., *Bolbitis serratifolia*) located within 20 cm of each other were counted as part of the same clump; otherwise, they were counted as separate individuals (*cf.* Costa 2006). For large-sized species, such as *Bactris major* and *Heliconia* spp., we determined clump formation by excavating the roots and checking for underground connectivity. We identified the species in the field when possible, using field guides for ferns, Marantaceae and Zingibelares (Zuquim 2008, Costa et al. 2011a, Costa et al. 2011b). For all species, we made botanical collections to confirm their identity at the Herbario del Oriente Boliviano in Santa Cruz, Bolivia.

To determine the soil properties, we collected seven soil samples, ~250g each, at intervals of 25 m within each plot, sampling mineral soil to a 40 cm depth. We created a compound soil sample per transect, from which we characterized soil color using the Munsell System. Soil samples were further analysed at the Centro de Investigación Agrícola Tropical (CIAT) in Santa Cruz, for measurement of the following chemical and physical variables: concentrations of N, P, exchangeable Ca^{2+}, Mg^{2+}, K^+ , Al^{+3}; pH; organic matter content (OM); total exchangeable bases (TEB); cation exchangeable capacity (CEC); base saturation; acidity; and soil texture. Soil texture (percentage of sand, silt, and clay) was measured using a Bouyoucos hydrometer; pH and electric conductivity was determined in 1:5 distilled water equilibrating for 90 min.; concentrations of exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+ were determined in 1 M ammonium acetate at pH 7. After extraction, Ca^{2+} and Mg^{2+} were measured with an Atomic Absorption Spectrophotometer; concentrations of K^+ and Na^+ were measured with a flame photometer; exchangeable aluminium (Al^{3+}) was measured in 1 M hydrochloric acid (HCl). Total P was determined by Olsen method; OM was determined by Walkley-Black method, and total N was measured using the Micro-Kjeldahl method (Barber and Cochrane 1993). TEB was defined as the sum of the most important exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+). CEC was calculated as the sum of TEB plus acidity. Base saturation was computed as the percentage of exchangeable cations or bases relative to total CEC.

We measured canopy openness to account for influence of light intensity on species distribution. Canopy openness was determined with a convex spherical densiometer at four cardinal points by two independent observers that held the densiometer at ~1 m above ground level. Measurements were strongly correlated between observers ($R^2 = 0.83$; $p < 0.001$). Canopy openness was measured every 5 m in each transect, and the 25 points were then averaged to give one estimate per plot (Englund et al. 2000, Paletto and Tosi 2009).

Data analyses

We explored overall variability across our plots using Principal Component Analysis (PCA) based on eight chemical variables (pH, concentrations of P, exchangeable Ca, Mg, K, Na, total N, and OM content) and three physical variables (percentage of sand, silt, clay), using the values from all 36 plots. Because variables had different measurement units, we standardized them by subtracting from the mean and dividing by the standard deviation. PCA was based on a correlation matrix.

Environmental effects on species composition

To evaluate the effect of ADE soils on species composition we selected a subset of edaphic variables considered as indicators of the presence of ADE soils (Glaser and Birk 2012). These variables, hereafter called ADE variables, are: pH, concentration of P and Ca, OM content, total N and presence of ceramics. To determine which factors drove understory species composition, we performed Redundancy Analyses (RDA) using the six ADE variables as well as UTM coordinates, light availability (canopy openness), and plot elevation as explanatory variables; and species composition with their abundances on each transect as dependent variables. Geographical UTM coordinates were included to account for the spatial distances among plots, given that the distance between plots varied from 0.25 to 8 km. Light availability was included, as light can vary among plots due to logging and application of silvicultural treatments (Peña-Claros et al. 2008); plot elevation accounted for topographical differences in La Chonta. RDA is a constrained ordination method, in which the ordination axes are linear combinations of all explanatory variables. RDA constrains the variability of the data set (species composition per transect) to these ordination axes, and tests the significance of this ordination with Monte Carlo Permutation tests. Two Monte Carlo Permutation tests were performed: the first one was used to determine the significance of the species composition variability explained by all canonical axes; the second was used to asses the significance of the contribution of each explanatory variable (Lepš and Šmilauer 2003). The latter analysis allowed us to select those independent variables that significantly explained species composition in the plots. We did four RDA analyses: one for the complete dataset (all 37 species); one for herbs (Commelinaceae, Costaceae, Heliconiaceae, Marantaceae, Poaceae, and Zingiberaceae); one for ferns; and one for palms. We evaluated these groups separately as they may respond differently to soil fertility (Costa 2006, Andersen et al. 2010a).

Environmental effects on species richness and abundance

Species richness per transect was modelled as a function of the six ADE variables, spatial location, plot elevation, and light intensity using multiple regression analysis based on generalized linear models (GLM). These models allow for not having non-linear combinations between expected values of response and explanatory variables, by including a non-linear link function. In our case, we used the logarithm as a link function for count data. We used backward multiple regression to evaluate significance of the explanatory variables that were standardized with mean 0 and variance 1, to be able to compare parameters estimated in the model. Regressions were performed for species richness of the whole community (all group of plants combined), for herbs (all families combined), and ferns, but not for palms, since only a total of six palm species were found. GLM were also used to assess the effect of ADE variables, spatial location, plot elevation, and light intensity on the abundance of individuals per transect for herbs (all families combined), ferns and palms. Palm abundance was included in the analysis because there were enough individuals in the plots to do the analyses.

Finally, to evaluate the response of individual species to ADE soils, we analysed species response curves. For each species that occurred in more than six plots (i.e., > 15 % of sample units: 11 herbs, 9 ferns and 5 palms) we determined response curves to pH, concentration of Ca and canopy openness. We tested only for pH and Ca because they are good indicators of the presence of ADE soils at La Chonta and those variables most influenced species composition, richness and abundance (see Results). We fitted linear and quadratic regressions for each explanatory variable, and selected for each species and for each variable the model with the lowest Akaike Information Criterion (AIC).

RDA and response curves of individual species were done using CANOCO 5 software (Ter Braak and Šmilauer 2012); paired t-tests and GLM were performed using Genstat 15.1 (VSN International Ltd).

Results

Nutrients and texture of ADE soils

The ADE soils were generally more fertile than non-ADE soils, as indicated by the PCA based on soil chemical and physical characteristics which explained 82% of the variation among plots. The first axis, which explained 60.9% of the variance, separated plots with high concentration of P, Ca total N, and high pH (with positive score values) from plots with lower quantities of these elements (with negative score values). ADE and non-ADE plots distribute continuously along the first PCA axis, indicating that there is a gradient of fertility between ADE and non-ADE soils (Fig. 2.1). The second axis explained 22.4% of the variance, but ADE and non-ADE plots were intermixed along this axis (Fig. 2.1).

General floristic observations

The studied groups consisted of 37 species: 14 ferns, 6 palms, and 17 species of herbs with 6 Heliconiaceae, 5 Marantaceae, 2 Costaceae, 2 Poaceae, 1 Zingiberaceae, and 1 Commelinaceae (see Appendix Table A2.2). Total abundance was 48,458 individuals, with 36,108 ferns, 10,194 herbs, and 2,156 palms. Among herbs, Heliconiaceae was the most abundant family with 6,747 individuals, whereas the most abundant species were the ferns *Bolbitis serratifolia* (29,386 individuals) and *Adiantum* cf. *pulverulentum* (6,152 individuals).

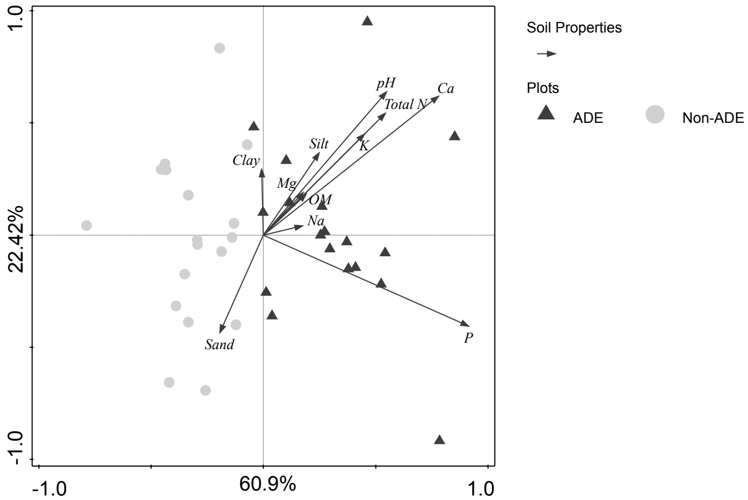

Figure 2.1 Results of Principal Component Analyses of soil parameters for Amazonian Dark Earths (ADE, 18 plots, black triangles) and non-ADE plots (18 plots, grey circles) in La Chonta, Bolivia**.** Arrows represent the environmental variables used in the analyses and their magnitude of importance in the ordination.

Effect of ADE variables on plant composition

Plant species composition was significantly influenced by soil and spatial variables (Fig. 2.2). RDA for all species combined and for each of the groups (herbs, ferns and palms) showed a consistent patter The first axis that explained a higher percentage of variation in all combined species, herbs and palms was associated with spatial variables, elevation or light, depending of the group that we evaluated, but this axis did not separate ADE from non-ADE plots (Fig. 2.2a, b, d). The second axis was associated with pH and variables related with fertility, such as concentration of soil exchangeable bases and total N. Interestingly, this axis dispersed ADE and non-ADE, suggesting that there is a gradient of fertility along ADE and non-ADE soils, mainly determined by differences in pH and Ca concentration, which are significantly associated with changes in the floristic composition of the plots (Table 2.1; Fig. 2.2a, b, d). Fern composition was better explained by soil variables, and ADE and non-ADE plots showed an intermingled distribution along the main axes (Fig. 2.2c).

The RDA showed that, when all species were combined, species composition was significantly explained by differences in pH, Ca concentration, spatial location and canopy openness (Table 2.1). When species groups were analysed separately, we found that canopy openness and concentration of Ca consistently explained the variance in all cases, but their contribution differed amongst groups. For ferns, Ca and soil pH explained 42% of the variation and for palms 20% of the variation in species composition. For herbs, Ca in the soil only accounted for 12% of the total variation. Spatial location significantly explained species composition of herbs and palms, whereas plot elevation significantly explained species composition of palms (Table 2.1).

Table 2.1 Results of a Redundancy Analyses (RDA) evaluating the effect of explanatory variables in species composition of all species together, and separately for angiosperm herbs (Marantaceae, Costaceae, Heliconiaceae, Poaceae), palms, and ferns. Data presented are the variation explained, R^2, followed by the p-value (in patentherises). The p-value is associated with the Monte Carlo permutation test that evaluated the null hypothesis of no correlation between the species composition and environmental variables. The level of significance for the individual environmental variables was $\alpha = 0.05$. Significant relations are shown in bold.

	Groups			
	All Species	Herbs	Ferns	Palms
All explanatory variables	**53.8 (0.002)**	**53.7(0.002)**	**42.8(0.012)**	**52.6(0.002)**
Individual variables				
pH	**9.3 (0.002)**	2.6 (0.19)	**7.1 (0.016)**	**5.3 (0.03)**
Total N (%)	**3.6 (0.05)**	**3.0 (0.14)**	2.7 (0.30)	**7.4 (0.01)**
P (mg kg^{-1})	1.9 (0.36)	1.0 (0.81)	3.5 (0.16)	2.9 (0.17)
Ca (cmol kg^{-1})	**4.8 (0.02)**	**6.5 (0.006)**	**15.0 (0.002)**	**5.2 (0.04)**
OM (%)	1.5 (0.65)	2.1 (0.31)	1.7 (0.58)	2.1 (0.32)
Ceramics	1.5 (0. 59)	1.6 (0. 50)	1.0 (0.9)	2.0 (0.38)
Canopy openness	**7.3 (0.004)**	**4.8 (0.03)**	**6.0 (0.02)**	**6.7 (0.02)**
X Coordinates	**18.6 (0.002)**	**25.9 (0.002)**	2.1 (0.48)	**6.9 (0.01)**
Y Coordinates	2.4 (0.16)	2.6 (0.20)	1.4 (0.71)	1.5 (0.58)
Plot elevation	2.8 (0.11)	3.4 (0.09)	2.4 (0.36)	**12.6 (0.006)**

Effect of ADE variables on richness and abundance

Species richness decreased when soil pH increased, both when all species were combined, and for the fern species alone (Table 2.2). Abundance of individuals was explained by soil variables only in the case of herbs and palms. Herb abundances significantly increased with

Ca concentration and canopy openness. Palm abundance was negatively affected by pH and positively affected by Ca concentration. Finally, fern abundances were negatively affected by canopy openness and spatial location (Table 2.2). These results are consistent with the RDA, which also indicated that spatial location, canopy openness, pH and concentration of Ca are the most important variables explaining species composition, richness and abundance.

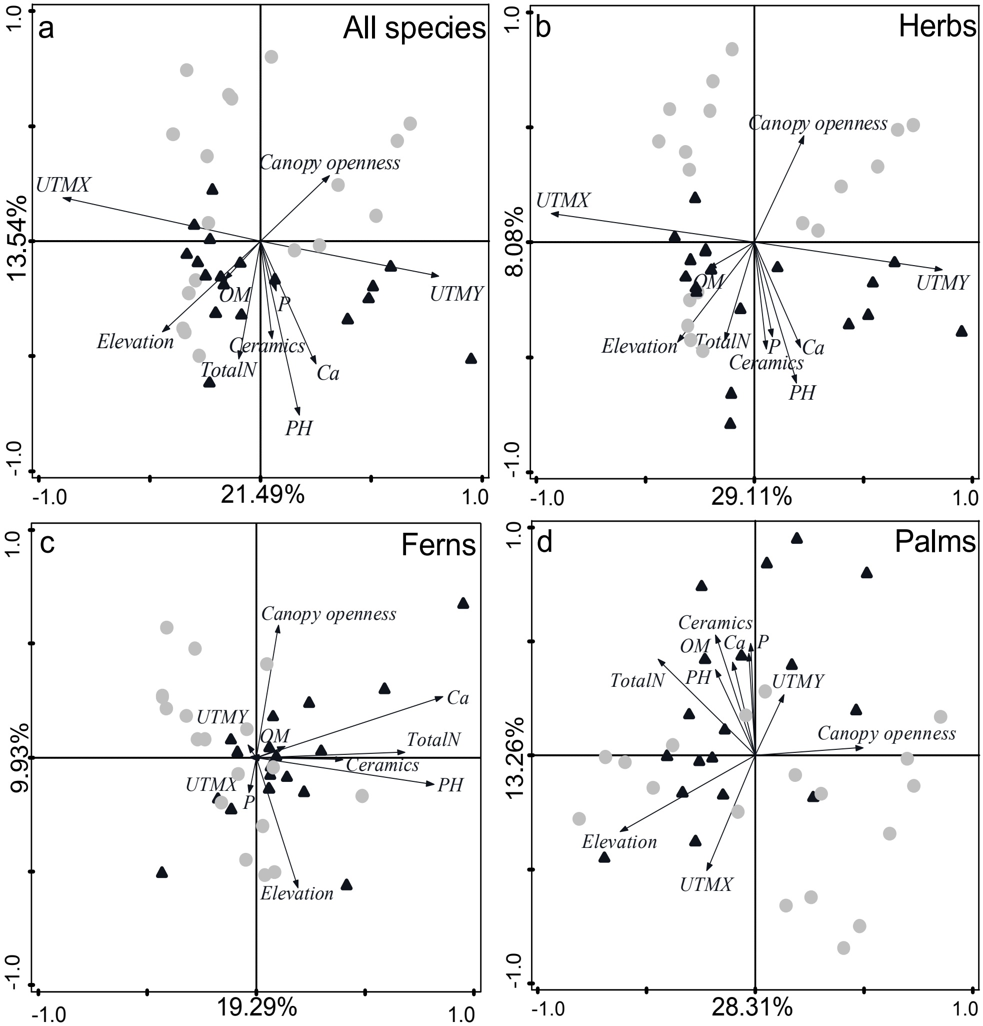

Figure 2.2 Biplots produced after the redundancy analyses, representing the distribution of the plots based in species composition and restricted to soil and environmental explanatory variables (Table 2.1). ADE plots are represented with black triangles and non-ADE plots are represented with grey circles. Arrows represent explanatory variables. Biplot of all understory species (a); of herbaceous species (b); of fern species (c) and of palms (d).

Individual species responses

We evaluated the response of the most frequent species to pH, Ca and canopy openness, since these variables consistently had significant effects on species composition, richness and/or abundance. Remarkably, of the 25 species evaluated, all but one (*Pharus latifolius*) showed a significant, although weak, response to pH and Ca (Appendix Table A2.3). Some palm species, e.g. *Astrocaryum murumuru*, the most abundant palm in our study site (1246 individuals), showed a type of bimodal response to Ca concentration and pH (Appendix, Table A2.3; Fig. 2.3f). More species responded to pH (12 out of 25 species) than to Ca concentration (7 species) (Fig. 2.3a-f) or canopy openness (6 species; see R^2 values in Appendix, Table A2.3). Many species were more frequent at lower pH, which could explain the negative relationship between pH and overall species richness (Table 2.2).

Table 2.2 Results of backward multiple regression models evaluating the effect of explanatory variables on species richness and abundance of individuals on each transect for all species combined, angiosperm herbs (Marantaceae, Costaceae, Heliconiaceae, Poaceae), palms, and ferns. Regression coefficients (a) of each explanatory variable that significantly fitted the model. For each model we present R^2, intercept (b) and significance level (p). Slopes (a) and intercepts (b) are represented in log scale given that a logarithmic function was used as the link function to fit the model ($LnY=b+a_1x+a_2x+...+a_nx$).

	Diversity			Abundance		
	All species	Herbs	Ferns	Herbs	Ferns	Palms
Model parameters						
R^2	0.17	0.07	0.44	0.40	0.51	0.52
b	2.72	1.95	1.40	5.57	6.82	4.01
p	0.007	0.069	<0.001	<0.001	<0.001	<0.001
Coefficients (a) of explanatory variables						
pH	-0.07		-0.21			-0.36
OM (%)						
Ca (cmol kg^{-1})				0.13		0.17
X Coordinates					-0.32	-0.59
Y Coordinates					-0.36	-0.29
Canopy openness				0.23	-0.42	
Plot elevation				-0.19		

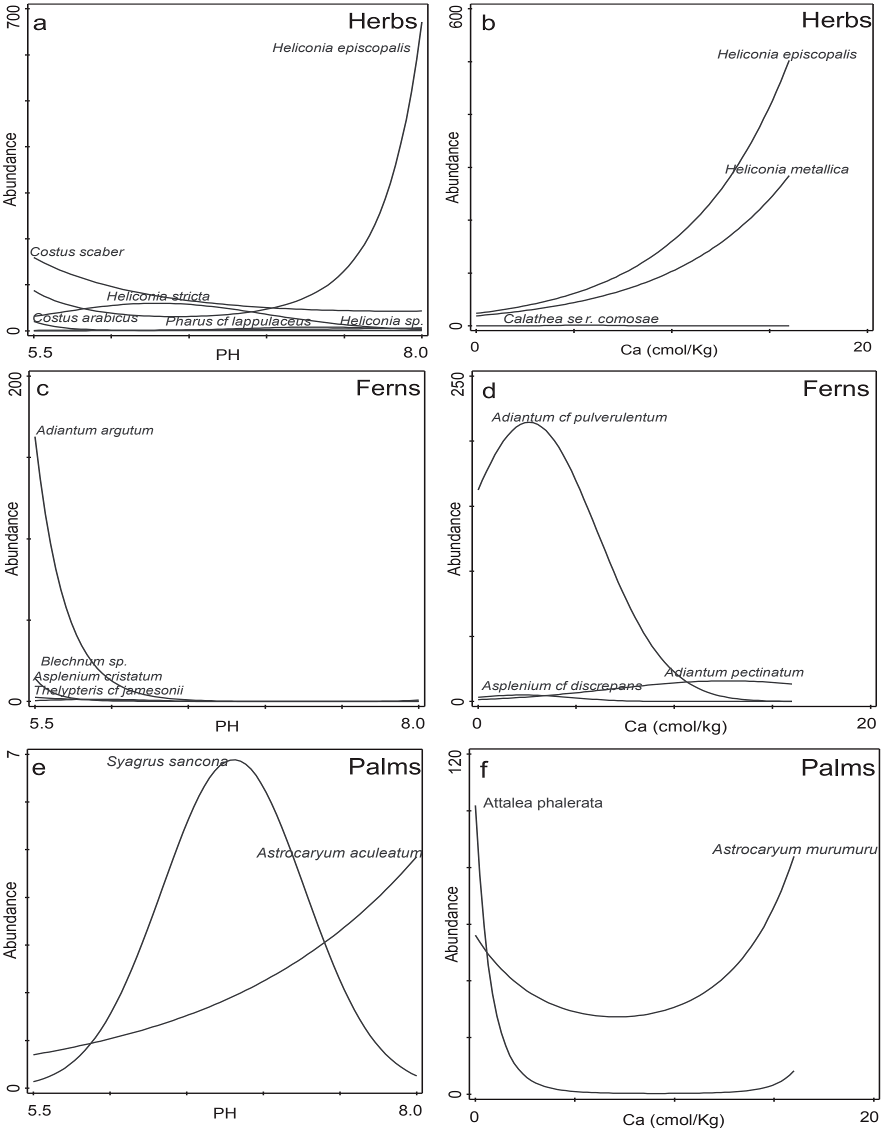

Figure 2.3 Responses of selected forest understory species to soil pH and Ca concentration in the soil at La Chonta, Bolivia. Modeled abundance values are shown for plots of $600m^2$: (a) herb species with the highest R^2 when soil pH was used as the independent variable, (b**)** herb species with the highest R^2 when Ca concentration in soil was used as the independent variable, (c) fern species with the highest R^2 when evaluated with soil pH as the independent variable, (d) fern species with the highest R^2 when Ca concentration in soil as the independent variable, (e) palm species with the highest R^2 when soil pH was used as the independent variable and (f) palm species with the highest R^2 with Ca concentration in soil as the independent variable. The values of R^2 are shown in Appendix. Table A2.2.

Discussion

We evaluated whether ADE soils have an effect on the understory plant community through changes in soil properties. Along the gradient from non-ADE to ADE soils in La Chonta, soil cations, P and total N increased as well as soil pH. Species composition also carried along this gradient, with species distribution associated with soil pH, Ca concentration and, to a lesser extent, total N. In general, species richness decreased with pH and abundance increased with nutrient levels. Consequently, we found that gradients in soil pH and Ca concentration generated by ADE presence were significantly related to gradients in composition, diversity, and abundance of the understory community.

Nutrients and texture of ADE soils

We hypothesized that past anthropogenic enrichment activities would have led to a higher fertility of ADE soils compared to non-ADE soils. We indeed found that 10 out of 12 chemical variables related to soil fertility were higher in ADE than non-ADE soils (Appendix Table A2.1). Most studies in the Amazonia have found large differences between ADE soils and surrounding soils in soil color, pH, organic matter content, CEC and concentrations of nutrients, such as P, Ca, N, Mg, K and Zn (Glaser and Birk 2012). Particularly, P and Ca concentrations are 100 times higher in ADE soils compared to the surrounding soils; however, variability in the concentration of P among and within ADE sites can be high (Lehmann et al. 2003b, Falcão et al. 2009). Interestingly, in La Chonta P and Ca concentrations were only 2.6 and 1.6 times higher in ADE compared to non-ADE soils, respectively. Similar results have been found in other regions of the Bolivian Amazonia (Hastik et al. 2013). These smaller differences between ADE and non-ADE are likely the result of higher background soil fertility levels in the study site. Given the geological history of this region (Southern Amazon basin) that includes the pre-existing Brazilian Shield and geological processes of sedimentation and erosion originated by the uplift of the central Andes in the Quaternary (Latrubesse et al. 2010), La Chonta soils vary widely in age.

We hypothesized that ADE and non-ADE soils are not so much two distinct groups but rather form the extremes of a continuum in soil fertility, which was indeed confirmed by the ordination analysis (Fig. 2.1). In several sites in Central and South of the Amazon basin, it has been suggested that inside archaeological ADE sites, variation in soil characteristics is better described by a fertility gradient that goes from the darker "center" region with higher fertility to lighter areas in the surroundings ending in the natural less fertile adjacent soils (Fraser et al. 2011, Schmidt et al. 2014).

Effect of ADE and other explanatory variables on species composition and species responses

We hypothesized that understory floristic composition is shaped by the presence of ADE soils. Our findings partially supported these hypotheses: understory floristic composition indeed responded to belowground resources that were indicators of ADE presence. However, to a lesser extent they also responded to aboveground resources (canopy openness) and were affected by spatial distribution of the plots (Table 2.1; Fig. 2.2). We found that, among the ADE variables, Ca concentration and pH accounted for around 25% of the total variation in species composition (Fig. 2.2). This was especially the case with fern species composition. Several studies on distribution patterns of ferns have shown the importance of soil exchangeable bases (Ca, Mg, K, Na) in explaining local (Costa et al. 2005, Tuomisto 2006) and regional (Zuquim et al. 2012, Zuquim et al. 2014) fern species composition, indicating that ferns specialize for, and partition, the exchangeable bases gradient (Tuomisto and Poulsen 1996, Costa et al. 2005).

We also found that Ca concentration, soil pH, and total N had a significant effect on palm species composition (Fig. 2.2d, Table 2.1). Similarly in Western and Central Amazon, palm composition was associated with exchangeable base concentration in the soil, both at local and meso-scales (Vormisto et al. 2004, Poulsen et al. 2006, Costa et al. 2009, Kristiansen et al. 2012), while in Panama palm species composition was most strongly associated with inorganic soil nitrogen (Andersen et al. 2010b). As for herbs, the effect of pH was not significant, and Ca in the soil had only a weak effect on species composition (Table 2.1). This result is in agreement with another study in Central Amazonia, where exchangeable base concentration in the soil explained a low percentage of the variance of herb species composition (Costa et al. 2005). In general, in the soil fertility gradient ranging from non-ADE to ADE soils, we found species of ferns and palms represented mainly by *Adiantum* cf *pulverulentum*, *Adiantum argutum* and *Attalea phalerata* in the least fertile extremes and herb species such as *Heliconia episcopalis* and *Heliconia metallica* in the most fertile extremes (Fig. 2.3).

Even at the restricted spatial scale of this study (< 10 km^2), geographical location of the plots had an effect on palm and herb species composition but not on fern composition (Table 2.1). The spatial effect on composition could be related to dispersal limitation, past human disturbance, and other environmental variables that we did not measure but that have a spatial pattern. Palms and herbs have probably a spatial component in their distribution because large palm seeds tend to disperse closer to the parent trees and many herb species studied here are clonal. On the other hand, fern spores are light and can disperse larger distances, and therefore a spatial effect on fern composition is not to be expected at this scale. Spatial location was also significantly correlated soil texture (sand: $r = -0.39$, $p = 0.01$; silt: $r = 0.40$, $p = 0.01$). Therefore, it is possible that herbs follow these differences in soil texture, as do species of the Marantaceae family in Central Amazonia (Costa et al. 2005), which may also help explain the spatial structure in herb composition.

Effect of ADE and environmental factors on species richness and abundance

We predicted that the increase in soil fertility of ADE soils would increase nutrient availability and plant growth, and hence species abundance and richness. We based our predictions on studies from relatively nutrient-poor sites in the Central and Western

Amazonia, where an increase in exchangeable bases concentration led to a higher density of fern species (number of species/area) and higher fern diversity (Tuomisto and Poulsen 1996, Costa 2006). In contrast, we found that species richness was not directly related with nutrients, but that it decreased with an increase in pH. We think of two possible, not mutually exclusive, explanations for this pattern. First, increases in soil pH are probably the result of Ca addition through human activities (bones) in our study area, as these two variables are highly correlated (Fig. 2.1; Pearson correlation, $r = 0.75$; $p < 0.001$). These changes are rather recent, preventing a high fern diversity in these relatively 'novel' environmental conditions. Second, competition with herbs and palms might affect fern diversity. In our plots, an increase in Ca concentration significantly increased the abundance of palms and herbs (Table 2.2): species such as *Astrocaryum murumuru* (Fig. 2.3f), *Heliconia epsicopalis* and *Heliconia metallica* (Fig. 2.3b) increased in abundance towards the more nutrient rich extreme of the gradient whereas ferns such as *Adiantum argutum* and *Adiantum* cf *pulverulentum* decreased with fertility (Figs. 2.3c, 2.3d). Polypod ferns, such as those found in our plots, can absorb N and P when they grow in fertile environments as some herbs do. However, their absorption of cations, particularly Ca, is significantly lower than for herbs (Amatangelo and Vitousek 2008). This could imply that in environments with increased amount of exchangeable bases in the soil, ferns would be able to take less advantage of the improved conditions compared to other groups of species.

Conclusions

We found that soil fertility differed along a gradient ranging from non-ADE to ADE soils, but that these differences were not as large as those found in other studies in Central Amazonia. Even on the relatively fertile natural soils at La Chonta, the presence of ADE (with increase of Ca) extends the available natural soil fertility along a gradient that influences the understory species composition, especially of ferns and palms. Past human modifications of the soil probably led to changes in the amount of exchangeable bases in the soil and in soil pH, which increased the abundance of some herbs and palms, and decreased fern diversity. We therefore conclude that ADE soils shape the forest understory community in this Bolivian forest.

Acknowledgements

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Appendix

Table A2.1 Soil characteristics of Amazonian Dark Earths (ADE) and non-Amazonian Dark Earths (non-ADE) found in a moist semi-deciduous forest in lowland Bolivia. Results of paired t-test are provided for each variable. Data provided are average ± standard deviation (SD). Variables in bold type are significantly different between ADE and non-ADE at $p < 0.05$.

Soil variable	ADE site	Non-ADE site
pH	**7.12 ± 0.39**	**6.59 ± 0.51**
Electric Conductivity (µS cm^{-1})	**88.83 ± 40.39**	**52.00 ± 29.06**
Acidity	**0.04 ± 0.06**	**0.11 ± 0.08**
Ca (cmol kg^{-1})	**6.17 ± 3.12**	**2.82 ± 1.46**
Mg (cmol kg^{-1})	1.09 ± 0.24	1.04 ± 0.27
Na (cmol kg^{-1})	0.09 ± 0.03	0.08 ± 0.02
K (cmol kg^{-1})	**0.25 ± 0.07**	**0.17 ± 0.06**
Total Exchangeable Bases (cmol kg^{-1})	**6.98 ± 2.63**	**4.73 ± 2.97**
Cation Exchange Capacity (cmol kg^{-1})	**7.02 ± 2.60**	**4.86 ± 2.92**
Base Saturation (%)	**99.33 ± 1.14**	**96.33 ± 2.85**
P (mg kg^{-1})	**12.94 ± 7.62**	**4.94 ± 1.70**
Total N (%)	**0.18 ± 0.04**	**0.13 ± 0.03**
OM (%)	2.48 ± 0.62	2.22 ± 0.42
Sand (%)	28.78 ± 3.88	27.44 ± 5.57
Silt (%)	56.28 ± 3.47	58.06 ± 4.85
Clay (%)	14.94 ± 2.13	14.50 ± 2.01

Table A2.2 List of herbs, palm and fern species and families found in the research plots of this study in "La Chonta" forestry concession, Guarayos Province, Bolivia.

Scientific name	Family
Astrocaryum murumuru	Arecaceae
Attalea phalerata	Arecaceae
Attalea glassmanii	Arecaceae
Astrocaryum aculeatum	Arecaceae
Bactris major	Arecaceae
Syagrus sancona	Arecaceae
Asplenium cf discrepans	Aspleniaceae
Asplenium cristatum	Aspleniaceae
Blechnum sp.	Blechnaceae
Dichorisandra hexandra	Commelinaceae
Costus scaber	Costaceae
Costus arabicus	Costaceae
Bolbitis serratifolia	Dryopteridaceae
Heliconia episcopalis	Heliconiaceae
Heliconia rostrata	Heliconiaceae
Heliconia stricta	Heliconiaceae
Heliconia metallica	Heliconiaceae
Heliconia x flabellata	Heliconiaceae
Heliconia sp.	Heliconiaceae
Calathea ser comosae	Marantaceae
Monotagma juruanum	Marantaceae
Calathea sp1	Marantaceae
Calathea sp2	Marantaceae
Calathea sp3	Marantaceae
cf Ischnosiphon	Marantaceae
Pharus latifolius	Poaceae
Pharus cf lappulaceus	Poaceae
Adiantum cf pulverulentum	Pteridaceae
Adiantum pectinatum	Pteridaceae
Adiantum argutum	Pteridaceae
Adiantum latifolium	Pteridaceae
Adiantum platyphyllum	Pteridaceae
Adiantopsis radiata	Pteridaceae
Pteris propinqua	Pteridaceae
Tectaria incisa	Tectariaceae
Thelypteris sp.	Thelypteridaceae
Thelypteris cf. jamesonii	Thelypteridaceae
Renealmia breviscapa	Zingiberaceae

Table A2.3 Results of models fitted on species abundance (response curves fitting) with frequencies higher than 15% of the plots. Soil pH, Ca concentration (cmol kg^{-1})and canopy openness were evaluated. From linear and/or polynomial functions, the model with the lowest AIC criteria was selected for each species. Cells with bold characters represent the explanatory variable that had the highest R^2 value for each species. Explanatory variables marked with (*) mean that the best fit of the models tested was a null model.

Species	Predictor	Model type	R^2	*F*	*p*
Palms					
Astrocaryum aculeatum	**pH**	**linear**	**0.08**	**11.5**	**0.002**
	Ca	quadratic	0.06	4.7	0.015
	Canopy Openness	linear	0.04	5.4	0.026
Astrocaryum murumuru	pH	quadratic	0.02	3.8	0.032
	Ca	**quadratic**	**0.13**	**27.6**	**<0.001**
	Canopy Openness	linear	0.08	33.1	<0.001
Attalea phalerata	pH	quadratic	0.45	96.9	<0.001
	Ca	**quadratic**	**0.49**	**107**	**<0.001**
	Canopy Openness	quadratic	0.19	41.3	<0.001
Bactris major	pH	quadratic	0.08	20	<0.001
	Ca	quadratic	0.06	14.7	0.007
	Canopy Openness	**quadratic**	**0.13**	**31.8**	**<0.001**
Syagros sancona	**pH**	**quadratic**	**0.25**	**21**	**<0.001**
	Ca	quadratic	0.16	13.7	<0.001
	Canopy Openness	quadratic	0.21	18.2	<0.001
Poaceae					
Pharus latifolius	pH	quadratic	0.02	2.2	0.127
	Ca	linear	0.01	3.2	0.082
	Canopy Openness	**quadratic**	**0.09**	**9.9**	**<0.001**
Pharus cf. *lappulacens*	**pH**	**quadratic**	**0.21**	**37.3**	**<0.001**
	Ca	quadratic	0.13	23.1	<0.001
	Canopy Openness	quadratic	0.19	32.8	0.001

Species	Predictor	Model type	R^2	*F*	*p*
Marantaceae					
Calathea ser. *comosae*	pH	quadratic	0.18	16.2	<0.001
	Ca	**quadratic**	**0.23**	**10.4**	**<0.001**
	Canopy Openness	quadratic	0.20	17.7	<0.001
Monotagma cf. *juruanum*	pH	linear	0.02	18.2	<0.001
	Ca	quadratic	0.18	67.7	<0.001
	Canopy Openness	**quadratic**	**0.32**	**121.7**	**<0.001**
Costaceae					
Costus arabicus	**pH**	**quadratic**	**0.48**	**18.7**	**<0.001**
	Ca	linear	0.20	16.2	<0.001
	Canopy Openness	quadratic	0.45	17.6	<0.001
Costus scaber	**pH**	**linear**	**0.24**	**294.9**	**<0.001**
	Ca	quadratic	0.16	116.5	<0.001
	Canopy Openness	quadratic	0.09	55.7	<0.001
Heliconiaceae					
Heliconia episcopalis	**pH**	**quadratic**	**0.38**	**773.7**	**<0.001**
	Ca	**linear**	**0.38**	**1571**	**<0.001**
	Canopy Openness	quadratic	0.26	524.5	<0.0011
Heliconia metallica	pH	quadratic	0.12	285.2	<0.001
	Ca	**quadratic**	**0.16**	**792.5**	**<0.001**
	Canopy Openness	quadratic	0.13	315.8	<0.001
Heliconia rostrata	pH	quadratic	0.04	7.7	0.002
	Ca	linear	0.01	10.5	0.002
	Canopy Openness	**quadratic**	**0.10**	**20.1**	**<0.001**
Heliconia stricta	**pH**	**quadratic**	**0.22**	**277.4**	**<0.001**
	Ca	quadratic	0.23	268.9	<0.001
	Canopy Openness	quadratic	0.03	48.2	<0.001

Species	Predictor	Model type	R^2	*F*	*p*
Heliconia sp.	**pH**	**linear**	**0.13**	**15.5**	**<0.001**
	Ca	quadratic	0.07	4.3	0.022
	Canopy Openness	quadratic	0.09	9.9	<0.001
Ferns					
Adiantum cf. *pulverulentum*	pH	quadratic	0.21	379.7	<0.001
	Ca	**quadratic**	**0.26**	**466.9**	**<0.001**
	Canopy Openness	quadratic	0.15	270.9	<0.001
Adiantum pectinatum	pH	quadratic	0.12	21.1	<0.001
	Ca	quadratic	0.15	26.8	<0.001
	Canopy Openness	**quadratic**	**0.18**	**33.0**	**<0.001**
Adiantum argutum	**pH**	**lineal**	**0.79**	**487.6**	**<0.001**
	Ca	quadratic	0.54	167.3	<0.001
	Canopy Openness	quadratic	0.41	128.2	<0.001
Asplenium cf. *discrepans*	pH	quadratic	0.08	8.6	0.001
	Ca	quadratic	0.16	18.0	<0.001
	Canopy Openness	quadratic	0.04	4.7	0.016
Asplenium cristatum	**pH**	**quadratic**	**0.40**	**6.7**	**0.004**
	Ca	linear	0.15	5.2	0.028
	Canopy Openness*	null			
Blechnum sp.	**pH**	**quadratic**	**0.47**	**9.3**	**<0.001**
	Ca	quadratic	0.40	7.5	0.001
	Canopy Openness*	null			
Bolbitis serratifolia	pH	quadratic	0.07	346.7	<0.001
	Ca	quadratic	0.08	404.9	<0.001
	Canopy Openness	**quadratic**	**0.43**	**2073**	**<0.001**

Species	Predictor	Model type	R^2	F	p
Pteris propincua	pH	linear	0.18	27.0	<0.001
	Ca	linear	0.17	25.8	<0.001
	Canopy Openness	**quadratic**	**0.25**	**19.1**	**<0.001**
Thelypteris cf. *jamesonii*	**pH**	**quadratic**	**0.43**	**9.2**	**<0.001**
	Ca	linear	0.15	6.4	0.016
	Canopy Openness	quadratic	0.38	8.1	0.001

Chapter 3

Effects of Amazonian Dark Earths on forest attributes and forest dynamics in a Bolivian forest

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Abstract

Heterogeneity of soil fertility across the Amazonian region comes from geological history and geomorphological processes. This heterogeneity drives forest structure and dynamics through the whole region, but at local scale the role of soils is unsolved. Amazonian Dark Earths (ADE) are anthropogenic soils related to the human occupation of the Amazonian region since pre-Columbian times; they contain high levels of P, Ca, and have high soil pH. ADE open an opportunity to test how increases in soil fertility could affect forest dynamics at local scales. In this study, we evaluated the effect of ADE on forest attributes such as standing basal area, tree liana infestation and successional composition defined by the relative presence of pioneers, long-lived pioneers, partial shade tolerant and shade tolerant species in the forest. We also evaluated the effect of ADE on components of forest dynamics (basal area growth, recruitment, and mortality). We found that soil fertility, most probably associated with ADE, weakly affected basal area, liana infestation and successional composition of the plots. Besides, it affected basal area of recruited trees and mortality of large trees. A higher soil pH increased recruitment of intermediate-size trees (20-40 cm in stem diameter) and decreased mortality of large trees (stem diameter > 40 cm). The most important effect of soils on forest dynamics, however, is through the effects of soil pH on initial basal area and successional composition that directly affected basal area growth of intermediate-size trees. In this Amazonian forest ADE, and soils in general, are not a strong driver of forest dynamics, and other environmental factors, such as light availability and liana infestation, play a stronger role.

Key words: Soil pH, Bolivia, forest dynamics, forest structure

Introduction

Soil is an important driver of vegetation structure and dynamics at different spatial scales (Paoli et al. 2008, Cleveland et al. 2011, Banin et al. 2014). For example, at large spatial scales across the Amazonian region, a soil fertility gradient runs from generally poor soils in the east to generally rich soil in the west with profound influences on forest dynamics (Phillips et al. 2004, Malhi et al. 2006, Quesada et al. 2009, Quesada et al. 2012). This east-west gradient in soil fertility is caused by a combination of the geological history of Amazonian soils and geomorphological processes (Hoorn et al. 2010, Quesada et al. 2010). On one hand, the older soils in the eastern and northern regions are heavily weathered and limited in nutrients but have good physical properties, such as deep soils and a texture that allows for good drainage. On the other hand, the younger soils in the western and southern regions have high bed rock-derived soil nutrient concentrations but poor physical properties, such as shallow soils and poor drainage (Quesada et al. 2011). The poor soils in the eastern Amazonian forest lead to slow tree growth, and less tree mortality related to deeper rooting on more profound soils, leading to slow stand turnover rates. On the contrary, the rich and shallow soils in the western Amazonian forests lead to faster tree growth and higher tree fall due to uprooting, giving rise to high stand-level turnover rates (Phillips et al. 2004, Quesada et al. 2012, Phillips and Lewis 2014). Among the soil variables, soil phosphorus (P), cation (Ca, K, Mg) concentration, soil nitrogen (N) and physical characteristics (soil depth, soil structure, topography) seem to be the main factors driving Amazonian forest dynamics (Quesada et al. 2012). Rich soils with higher amounts of total and plant available phosphorus (P) increased net primary productivity of aboveground biomass (leaves, branches, stems and volatile compounds) and below-ground biomass (fine roots, coarse roots and root exudates) in 9 1-ha plots across the Amazonian region (Aragão et al. 2009). Besides, these east-west soil gradients also affect forest structure (Quesada et al.2009) and species composition (ter Steege et al. 2006, Quesada et al. 2012). Aboveground biomass (AGB) is higher in the eastern poor soils regions, whereas lower AGB is more common in the rich soils of the west (Malhi et al. 2006, Quesada et al. 2009). This pattern seems to be related to tree species composition from north-east to south-west Amazon basin. North-eastern forests have more shade-tolerant species with higher wood density and larger seeds, while south-western forests have more light-demanding species with lower wood density and smaller seeds (ter Steege et al. 2006, Quesada et al. 2012).

At smaller spatial scales, the strength of soils in explaining forest dynamics and forest structure decreases drastically. Comprehensive studies in Bolivian lowlands, including the Amazonian region of the country, show that rainfall is a stronger driver of forest structure and dynamics and of tree species composition than soil variables. For example, basal area growth was negatively related to soil concentration of Ca, P and percentage of N (Toledo et al. 2011b); tree and palm density and liana infestation were negatively related with soil fertility (Toledo et al. 2011c); and finally, calcium (Ca) and P in the soil affected tree species composition after having accounted for the major influence of rainfall (Toledo et al. 2011c).

Similarly, small scale studies on the island of Borneo show that a combination of climatic and soil variables affect forest structure and dynamics, and especially basal area and AGB were positively related with soil fertility (Slik et al. 2010). At more reduced scales, around 15 km^2, soils also play an important role on forest dynamics and structure in the Paleotropics. In a forest in Borneo, basal area and AGB was affected by soil nutrients (P and N) and the effect was higher for large trees (DBH > 120 cm) and lower for intermediate-size trees (60-90 cm in DBH) (Paoli et al. 2008). In a forest in western Borneo, basal area and AGB growth of trees > 40 cm in DBH were positively correlated with total soil P and cation exchange capacity (CEC) (Paoli and Curran 2007). In the Neotropics few studies have found relations between nutrients and forest structure at local scale. In central Amazonian forest AGB increased with increase in soil N and percentage of clay (Laurance et al. 1999); in Reserva Duke, in the central Amazonian region, AGB was higher in clayey soils that are located in high elevations (de Castilho et al. 2006); in the western Amazonian region, in the reserve of Cocha-Cachu reserve, Peru, trees > 30 cm DBH had higher AGB in more fertile compared with less fertile soils (DeWalt and Chave 2004). Forest structural aspects and the association with soil characteristics can be related to forest composition if, for example, large emergent tree species are dominant in sites with high nutrients (Paoli et al. 2008). Additionally, endogenous processes like gap formation (Ruslandi et al. 2012) and human perturbation could increase tree light availability that influences forest structure and dynamics (Peña-Claros et al. 2008) and even forest composition (Carreño-Rocabado et al. 2012, Bourland et al. 2015). Besides, the management of man-useful trees through cultivation can influence forest composition (Levis et al. 2012).

One important structural component of tropical forest are lianas (Schnitzer and Bongers 2002, Schnitzer et al. 2015). Additionally, lianas can have an effect of forest dynamics through its influence on tree mortality (Phillips et al. 2005). Therefore, environmental factors that drive lianas can indirectly affect forest dynamics. The effect of soils on lianas seems not to be very strong and in many cases it depends on the scale. Across the tropics, lianas are influenced by rainfall and climate seasonality (DeWalt et al. 2010, DeWalt et al. 2015), which can (at least partly) be explained by higher liana tolerance to drought (Schnitzer 2005). Nevertheless, patterns at smaller spatial scales are different and even contrasting. Studies focussing on Neotropical ecosystems found very weak relationship between climatic and soil variables in liana density and liana basal area (Van Der Heijden and Phillips 2008); but in Cocha-Cachu, Peru, liana density decreased in richer soils (DeWalt and Chave 2004). Recent detailed studies suggest that liana densities were not related with soil nutrient variables but with water availability in the dry season and with gap formation (Dalling et al. 2012, Schnitzer et al. 2012) although few liana species are associated with richer soils in Barro Colorado Island, Panama suggesting species specific responses (Dalling et al. 2012).

The main origin of P in the soil is the weathering of the parental material (Quesada et al. 2010). Nevertheless, in the Amazon basin, there are soils from anthropogenic origin that can have high levels of P. These soils are called Amazonian Dark Earths (ADE) ('T*erra*

Preta' in Portuguese) (Sombroek 1966, Glaser and Birk 2012), which is a type of Anthrosol characterized by a thick dark or gray top layer with presence of ceramics indicating past indigenous settlements (Sombroek 1966, Woods and Glaser 2004). ADE are the product of kitchen remains, such as garbage deposition, over thousands of years (Schaan et al. 2009). ADE patches are found throughout the Amazonian region, accounting for *ca*. 3.2% of the area (McMichael et al. 2014). ADE patch sizes vary from less than 2 to more than 100 ha, with most of them, around 80%, being *ca.* 2 ha (Winklerprins and Aldrich 2010). They possess higher concentrations of total and available P and Ca, higher total N (Lehmann et al. 2003b), and higher concentrations of charcoal and C (Glaser et al. 2003) than the surrounding Ultisols (Acrisols in WRB -world reference base for soil resources) and Oxisols (Ferrasols in WRB) in most of the Amazonian region (Falcão et al. 2009). When ADE soils are present in a given area, they form a gradient of fertility from the center of the ADE patch to the surrounding natural soils (Fraser et al. 2011, Costa et al. 2013). Some of the main benefits in soil properties of ADE are related to the addition of charcoal that leads to more stable organic matter, high nutrient levels and probably higher soil water retention capacity (Glaser et al. 2002). Moreover, the addition of organic waste leads to increase in nutrient concentrations (P, N, and Ca) (Glaser 2007), with Ca increasing the concentration of exchangeable bases and soil pH, which in turn also increases the availability of other cations. ADE provide, therefore, important human legacies, that can have potentially long-lasting impact on the vegetation, but few studies have related ADE with forest structure and dynamics. Secondary forests (~30 y old) that had been growing on ADE had lower tree density than secondary forest growing in non-ADE soils especially at early stages of the forest succession (Junqueira et al. 2010). Comparative studies across the Amazonian forest showed that net primary productivity (NPP) of one forest plot established on ADE had higher NPP; notably this increase was due to higher below-ground biomass and not to above-ground biomass (Aragão et al. 2009). Similarly, Doughty et al. (2014) found an increase in gross primary productivity GPP) and high rates of fine root production on a forest growing on ADE compared with a forest growing on non-ADE. These local-scale studies evaluated the effects of ADE on some forest structural variables and NPP, but they did not consider the underlying demographic processes that shape forest dynamics such as tree growth, tree recruitment and tree mortality.

Conceptually, at local scales, abiotic resources affect both forest structural attributes and forest dynamics, while the forest attributes, on their turn, affect forest dynamics (Fig. 3.1a). Basal area is affected by abiotic factors such as soils (Paoli et al. 2008) and light through forest perturbation (Saldarriaga et al. 1988, Peña-Claros et al. 2008). Also species composition may be driven by abiotic factors as species requirements can parallel a local gradient in resource availability (Paoli et al. 2006). At the same time, soils and light may positively affect liana infestation (Dalling et al. 2012) (Fig. 3.1a), while liana infestation generally negatively affects basal area through increased tree mortality (Phillips et al. 2005, Peña-Claros et al. 2008, van der Heijden et al. 2015) (Figs. 3.1a, 3.1b). Demographic processes that determine forest dynamics can also be affected, at local scales, by abiotic and biotic factors. Soil fertility (Paoli and Curran 2007) and light (Peña-Claros et al. 2008) are shown to positively affect forest growth and recruitment (Fig. 3.1b). Species composition may increase forest dynamics through fast growing species that also die faster (Phillips and Lewis 2014) (Fig. 3.1b).

ADE provide an unique opportunity for testing if increases in soil nutrients can influence forest dynamics and structure at small scales. Therefore, in this study we evaluated the effect of ADE on forest attributes and forest dynamics of a lowland Bolivian forest. We predicted that the presence of ADE increases soil fertility affecting positively forest attributes, such as liana infestation and standing basal area, and that sites with higher soil fertility increase the proportion of species with more resource acquisitive strategies, such as pioneers (Fig. 3.1a). Furthermore, increased fertility of ADE soils positively affects forest dynamics by increasing tree growth (measured as basal area growth) and recruitment, and by increasing mortality of fast growing species (Fig. 3.1b –left side–). Forest attributes are directly affected by soils but, simultaneously, forest attributes on their turn affect forest dynamics. Therefore, we predict that liana infestation has a negative effect on basal area growth and recruitment (basal area recruitment), and a positive effect on mortality (basal area mortality) (Fig. 3.1b –right side–). Additionally, we predict that forest successional composition is related with basal area growth, basal area recruitment and basal area mortality, with the more dynamic plots having more fast growing species (Fig. 3.1b –right side–). Finally, we predict presence of ceramics to be directly related to species composition, forest dynamics and presence of lianas, as the ceramics reflect human perturbation that created areas with higher light, and/or enriched the forest with useful species (Fig. 3.1b).

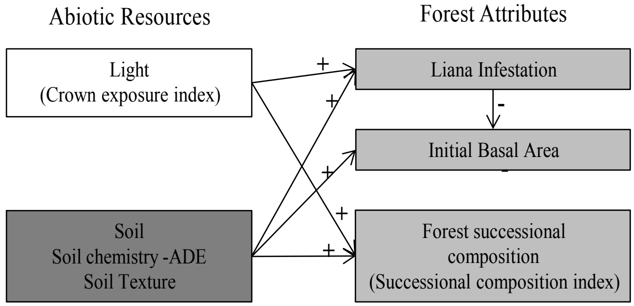
Methods

Study site

The study was conducted in the 100,000 ha forestry concession of Agroindustria Comercial La Chonta, Guarayos province, Bolivia (15º47'S, 62º55'W). Mean annual temperature in the area is 24.3 °C and annual rainfall is 1580 mm (data from 2000 - 2006 from La Chonta sawmill) with a dry season (< 100 mm/mo) from May through September (Peña-Claros et al. 2012). The study site is located on the South-West border of the Brazilian Precambrian Shield. Soils at La Chonta are heterogeneous, they are a mosaic of old, more nutrient depleted soils from the Brazilian Precambrian Shield (Navarro and Maldonado 2002) and more fertile soils that are the product of sedimentation and erosion originated from the uplift of central Andes (Latrubesse et al. 2010). Furthermore, La Chonta contains anthropogenic soils that account for about 20 percent of a studied area of 216 ha (Paz-Rivera and Putz 2009).

The forestry concession of La Chonta was established in 1974. Currently, they harvest around 3 trees/ha (7.7 m^3/ha) belonging to 17-19 tree species. During its first 20 years, the concession logged only mahogany trees (*Swietenia macrophylla*) and tropical cedar (*Cedrela odorata*). Before this time there was no evidence of forest management in the area. Nevertheless, the presence of ADE soil with pieces of charcoal and pottery of ~480 to 250 years B.P. indicates that there were indigenous settlements in the area (Paz 2003).

a) Effects of Abiotic Resources on Forest Attributes



b) Effect of Abiotic Resources and Forest Attributes on Forest Dynamics

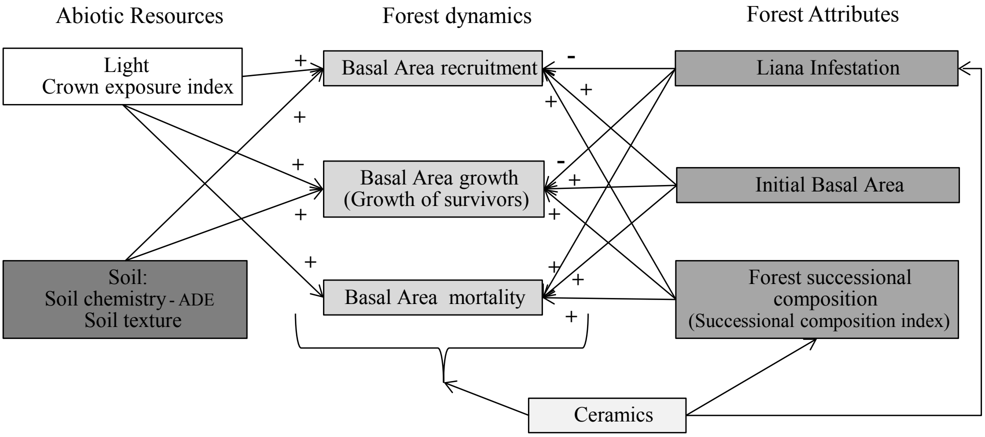


Figure 3.1 Conceptual framework showing **a)** the effects of abiotic resources (light, soils) on forest attributes: liana infestation, initial plot basal area and forest successional composition (successional composition index). Successional composition index is based on the ecological guilds of the species present in the plots that vary from shade tolerant to pioneer species (see *Methods*). Although our main interest is the effect of soils (both chemical and physical characteristics), we include light in our framework given its strong effects on forest dynamics. **b)** the effects of abiotic resources (light, soil chemistry and soil texture) on stand basal area growth (based on the trees surviving the whole monitoring period of 8 to 10 years), stand basal area recruitment (based on the basal area of the recruited trees that survive until the last census) and basal area mortality (based on the basal area of the trees sampled at plot establishment that died from natural death) –left side of the figure. The right side of the figure considers the effects of forest attributes (structure and successional composition) and ceramics (as the absolute proof of human occupation of the forest) on forest dynamics (basal area growth, basal area recruitment, basal area mortality).

The forest at La Chonta is classified as a semi-deciduous tropical moist forest (Peña-Claros et al. 2012). Floristic comparisons of several lowland forests in Bolivia show that the forest at La Chonta is transitional between the forest of the Northern Amazonian region and the Southern Chiquitano dry forest (Toledo et al. 2011a). The forest has, on average, 367 stems (>10 cm diameter at breast height [DBH]) and 59 species per ha (Peña-Claros et al. 2012), an average tree height of 27 m, and approximately one third of the canopy species lose their leaves in the dry season (Markesteijn et al. 2010).

Permanent plots design and census

This research was carried out in twelve large-scale 27-ha permanent sample plots established by the Bolivian Forest Research Institute (IBIF, due to acronym in Spanish) that are part of the Long-term Silvicultural Research Program (LTSRP) (see Peña-Claros et al. 2008)for details). Within each plot the mapping and measuring of trees was done using a nested design: trees with a diameter > 40 cm (measured at 1.3m aboveground, DBH) were measured in the whole (27ha) plot; trees with a diameter between 20 and 40 cm were measured in half of the plot (13.5ha); and trees with a diameter between 10 and 20 cm were measured in four 1-ha subplots (Peña-Claros et al. 2008). Establishment of the plots was done between September 2000 and December 2001. After plot establishment, plots were logged as part of the treatments of the research program above mentioned. Plots were then measured 1 year after establishment and then around every two years until November 2011.

Soil sampling

To map the presence of ADE soils in the twelve 27-ha plots, we sampled the soil at each intersection of a 50x50 m grid the soil with an auger to 40cm depth. For each sample, we described the soil color with Munsell Soil Color Charts and recorded the presence of ceramics or charcoal as evidence of past human occupation. We then collected around 250 g of soil to make posterior chemical and physical analyses. We gathered around 130 samples per plot. Once in the lab, we assessed soil pH for all samples by taking 25 mg of soil per sample and combining it with 10 ml of distilled water; we then measured pH with a Thermo Fisher Scientific Orion start meter (Beverly, MA, USA).

In La Chonta, ADE creates a gradient in soil characteristics, particularly in soil pH (Quintero-Vallejo et al. 2015). As we wanted to characterize this gradient on every plot but could not analyse all samples due to economic restrictions, we selected 20-40 soil points per plot to perform further chemical and physical analyses, providing a total of 400 soil points. On each plot we selected soil points in such a way that half of them had pH higher than 7, dark color (black or very dark brown color, mainly 7.5YR 2.5/1, 7.5YR 2.5/2 and 7.5YR 2.5/3 in the Munsell system), and ceramics were present; whereas the other half of the soil points had pH lower than 7, lighter soil color (brown, yellowish brown or reddish brown, mainly 7.5YR 4/3, 7.5YR 4/4, 7.5YR 5/4, 5YR 5/3), and no ceramics. The following chemical and physical variables were measured at the Centro de Investigación Agrícola

Tropical (CIAT) in Santa Cruz (Bolivia): concentrations of N, total P, exchangeable Ca^{2+}, Mg^{2+}, K^{+}, and Al^{+3}; pH; organic matter content (OM); total exchangeable bases (TEB); cation exchangeable capacity (CEC); base saturation; and soil texture (percentage of sand, silt, and clay (for extraction methods see Annex 1).

Tree selection and forest dynamics variables

To study forest dynamics we used the 400 soil points, located in the twelve 27 ha main plots, for which we had soil chemistry and texture information. From the point where the soil sample was taken, we established a circular plot with a radius of 25 m and selected the trees that were within this distance. These circular plots are referred as subplots hereafter. Because of the nested plot design (as described above), the subplots did not contain trees of all size classes. Therefore, we divided the tree dataset as follows for further analysis: trees > 40 cm in DBH (in 387 subplots), hereafter referred to as large trees (which are all in the canopy); trees between 20 - 40 cm in DBH (in 214 subplots), referred to as intermediate-size trees (which are mostly in the sub-canopy); and trees between 10 - 20 cm in DBH (in 99 subplots), referred to as small trees (which are mostly in the understory). This subdivision also allows to evaluate whether trees in different ontogenetic stages respond differently to abiotic resources and forest attributes. We determined forest dynamics using changes in basal area in a period of 8 to 10 years depending on the date of plot measurement. We used basal area instead of number of stems to account for soil points located in the borders of the 27-ha plots, for which we needed to use a measurement that allowed us to correct for subplot area. We divided the forest dynamics into three underlying components all expressed in $m^2\ ha^{-1}\ y^{-1}$. For each subplot basal area growth was estimated by subtracting the final basal area (i.e. at the time of the final census) from the initial basal area (i.e. at the time of plot establishment) of the trees that survived from the establishment until the last census, divided by the number of years between the plot establishment and the final census for each plot. Basal area recruitment was determined by the sum of the basal area of all recruited trees on each subplot from the first census until the last census, divided by the time that passed between these two censuses. Basal area mortality was determined by the sum of the basal area of all trees that died from the first until the last census and dividing it by the time passed between these censuses. Subplot basal area was determined by adding the basal area of all trees that belonged to the subplot ($m^2\ ha^{-1}$). Because we were interested in natural dynamics, we excluded trees that were logged or killed during logging operations or due to the application of silvicultural treatments, or post logging mortality (caused by profound damage during the logging operations). We also excluded trees that died due to a wildfire in 2004 that affected partially two of the 27-ha plots.

Light, forest structure and forest composition

Forest dynamics can be affected by light, liana infestation and forest composition (Schnitzer and Bongers 2002, Peña-Claros et al. 2008, Quesada et al. 2012). We characterized light received by each tree using the tree crown exposure index, which is ranked from 1 to 5 (Dawkins and Field 1978): 1 = no direct overhead light and little lateral light; 2 = no direct overhead light but moderate or substantial lateral light; 3 = vertical light on part of the crown; 4 = vertical light on the whole crown; and 5 = exposed emergent crown. To calculate a crown exposure at the plot level, we weighted the crown exposure index of each tree by its basal area and divided this amount by plot basal area as follows: $CEI_p = \frac{\sum_{i=1}^{n}(CEI_i \times BA_i)}{\sum_{i=1}^{n} BA_i}$, where CEI_p is the crown exposure index per plot p, CEI_i is the crown exposure index of tree i; and BA_i the basal area of tree i.

Liana infestation per subplot was determined using a liana infestation index for every tree that goes from 1 to 4 as follows (Alder and Synnott 1992): 1 = no lianas; 2 = lianas on stem; 3 = lianas on stem and crown; and 4 = lianas completely covering the crown. To determine the liana infestation per subplot we used the following equation: $LII_p = \frac{\sum_{i=1}^{n}(LI_i \times BA_i)}{\sum_{i=1}^{n} BA_i}$, where LII_p is the liana infestation index per plot p, LI_i stands for liana infestation of tree i, and BA_i stands for basal area of tree i. Liana infestation index range from 0% (stand biomass has not liana infestation) to 100% (stand biomass is completely infested by lianas). Additionally, we determined the successional composition index of every subplot using information on the ecological guild for each tree as follows: 1 = shade tolerant species; 2 = partial-shade tolerant species; 3 = long-lived pioneer species; and 4 = (short-lived) pioneer species (Jardim et al. 2003, Mostacedo et al. 2003, Justiniano et al. 2004, Poorter et al. 2006). We then calculated a successional composition index per plot using the equation: $SCI_p = \frac{\sum_{i=1}^{n}(EG_i \times BA_i)}{\sum_{i=1}^{n} BA_i}$, where SC_p is the succession composition per plot p, EG_i stands for the ecological guild of every tree i, and BA_i stands for basal area of tree i. We scaled liana infestation and successional composition index from 0 to 100%. For successional composition index values can range from 0% (stand biomass is fully represented by shade tolerant species); 33.3 (stand biomass represented mostly by partial-shade tolerant species); 66.6% (stand biomass represented mostly by long-lived pioneer species); and 100 (stand biomass is represented by short-lived pioneer species). Crown exposure (CEI_i), liana infestation (LI_i), and basal area (BA_i) per tree were averaged over all censuses from the establishment of the plots until the last census (2011) before calculations of the indices.

Data analysis

To determine the variables that affected forest attributes (initial basal area, liana infestation and successional composition, see Fig. 3.1), we fitted a series of linear mixed effects models with all possible combinations of the explanatory variables (all-subset combinations). On those models, explanatory variables were soil chemical variables that identify ADE such as soil pH, concentration of Ca, P, N, or OM content (hereafter called soil chemistry variables); sand, and clay content (soil texture variables); light (crown exposure index); and by the presence of ceramics on the sampled soil. Presence of ceramics was included since it indicates the human occupation of the forest. In the mixed models we used plots (12) nested in blocks (3) as random factors, to account for spatial correlation of subplots belonging to the plots and for internal variability of the plots due to the IBIF-research treatments. Since we found a strong correlation between pH and Ca concentration in the soil (Pearson correlation = 0.76), we excluded Ca from the analyses to avoid effects of collinearity. Liana infestation was a response variable, nevertheless we included this as explanatory variable in modelling the initial tree basal area since lianas can have a negative effect on tree growth and increase tree mortality. Consequently, liana infestation is expected to reduce the initial tree basal area (Fig. 3.1). Besides, we fitted a series of linear mixed effects models to evaluate if forest dynamics (basal area growth, basal area recruitment and basal area mortality) were affected by soil chemistry, soil texture, light, presence of ceramics, liana infestation and successional composition.

From all possible models produced, we performed model selection by choosing those models that differed less than 2 Akaike's Information Criterion (AIC) units from the best model after they were ranked from the best to the worst according to the AIC score. We averaged the selected models (e.g. 4 or 5 models) to pick the explanatory variables with higher relative importance value (maximum 1), which is the sum of the *Akaike weights* (Burnham and Anderson 2002) of the models that include the explanatory variable is present (Barton 2012). We run the model with the variables that showed the highest importance value to determine if their regression coefficients were statistically significant at level of $\alpha=0.05$. In cases where explanatory variables did not have significant regression coefficients, we dropped them to attain a simpler model, and we compared models to test whether the resulting model was significantly different from the previous one. We fitted the final model (i.e. the simpler model, which explanatory variables had statistically significant regression coefficients) and we used restricted maximal likelihood estimation (REML) to determine regression coefficients. We calculate a pseudo-r^2 for each model, squaring the Spearman correlation coefficient between the fitted vs. the empirical data. Models for each response variable were fitted for the tree different tree size classes: trees > 40 cm in DBH, trees between 20 - 40 cm in DBH and trees 10-20 cm in DBH.

Modelling procedures and statistical analyses where performed in R (version 3.1.2), packages nlme, version 3.1.117 (Pinheiro et al. 2014) and MuMIn, version 1.10.5 (Barton 2012), using the functions *lme*, *dredge* , *model.avg*, and *anova*.

Results

Stand basal area was on average 9.0 ± 7.0 $m^2 ha^{-1}$ (mean ± SD) for large trees (DBH > 40 cm), 6.1 ± 2.5 $m^2 ha^{-1}$ for intermediate-size trees (20 - 40 cm in DBH), and 2.9 ± 1.8 for small trees (10-20 cm in DBH). In general, large trees had higher liana infestation index (59.7 ± 22.6 %) than small trees (43.9 ± 20.7 %). Additionally, the community of large trees had larger proportion of (long-lived) pioneers (average SC = 45.1 ± 16.4%), the intermediate-size tree community contained mostly partial shade tolerant (average SC = 33.7 ± 17.2%), whereas the small tree community was mainly composed of (partial) shade-tolerant species (average SC = 24.5 ± 13.42%).

Soil variables that are related with ADE and soil texture varied widely throughout our sampling area (324 ha). Ca, P and OM varied most strongly amongst subplots (Table 3.1); Ca and OM varied 100-fold and P 70-fold. The higher variability of P and Ca (which are indicators of ADE soils in the Amazonian region) plus the presence of ceramics indicate the presence of ADE soils in our study site (Table 3.1).

Table 3.1 Soil properties of 400 soil samples (0 - 40 cm depth) taken in twelve 27-ha plots in lowland tropical forest of La Chonta, Bolivia. Average, standard deviation (SD) and range (minimum to maximum) are given. Soil samples were taken to represent the whole gradient in soil fertility that is present in La Chonta from non-Amazonian Dark Earths to Amazonian Dark Earths). CEC=cation exchange capacity, TEB=total exchangeable bases, OM=organic matter.

Soil Variable	Average	SD	Range
Soil pH	6.70	0.89	(4.8 - 8.3)
Ca (cmol kg^{-1})	4.60	4.09	(0.2 - 22.8)
K (cmol kg^{-1})	0.21	0.15	(0.04 - 1.2)
Na (cmol kg^{-1})	0.05	0.05	(0 - 0.84)
Mg (cmol kg^{-1})	1.06	0.81	(0.1 - 9)
TEB (cmol kg^{-1})	5.69	4.51	(0.5 - 29.9)
P (mg kg^{-1})	12.03	11.92	(1 - 79)
N (%)	0.13	0.06	(0.01 - 0.37)
OM (%)	2.05	1.70	(0.28 - 33.1)
Sand (%)	58.74	9.45	(8 - 81)
Clay (%)	13.39	4.89	(0 - 38)
Silt (%)	27.90	7.72	(8 - 73)

Effects of soil and light on forest attributes

After model selection, the ‘best’ models that explain forest attributes had in general low predictive power determined by a low *Akaike weight*, which is a measure of predictive power of the model selected as the best (Burnham and Anderson 2002) (see Appendix, Table A3.1, for further details); and the models with significant explanatory variables had in general low pseudo-r^2 values, especially those that included soil variables (Table 3.2). In general, soil chemistry and soil texture affected forest attributes in all tree size categories differently (Fig. 3.2a, b, c). For large trees, soil pH had a negative effect on initial basal area and successional composition index (Fig. 3.3a, d), while soil N had a negative effect on liana infestation (Table 3. 2; Fig. 3.3f). For intermediate-size trees, soil pH had a positive effect on initial basal area and a negative effect on the successional composition index (Table 3.2; Fig. 3.3b, e), while soil P had a negative effect on initial basal area (Fig. 3.3c). For small trees, percentage of clay and sand negatively affected initial basal area and liana infestation simultaneously; although clay was not statistically significant it was selected in the most parsimonious model (Table

3.2; Fig. 3.2c; Appendix). Additionally, light affected forest attributes differently for each size category. For large trees, light had a negative effect on liana infestation and a positive effect on successional composition index. For intermediate-size trees, light had a positive effect on initial basal area, liana infestation and successional composition index (Fig. 3.2 b). For small trees, light positively affected liana infestation and successional composition index, but not initial basal area (Fig. 3.2c).

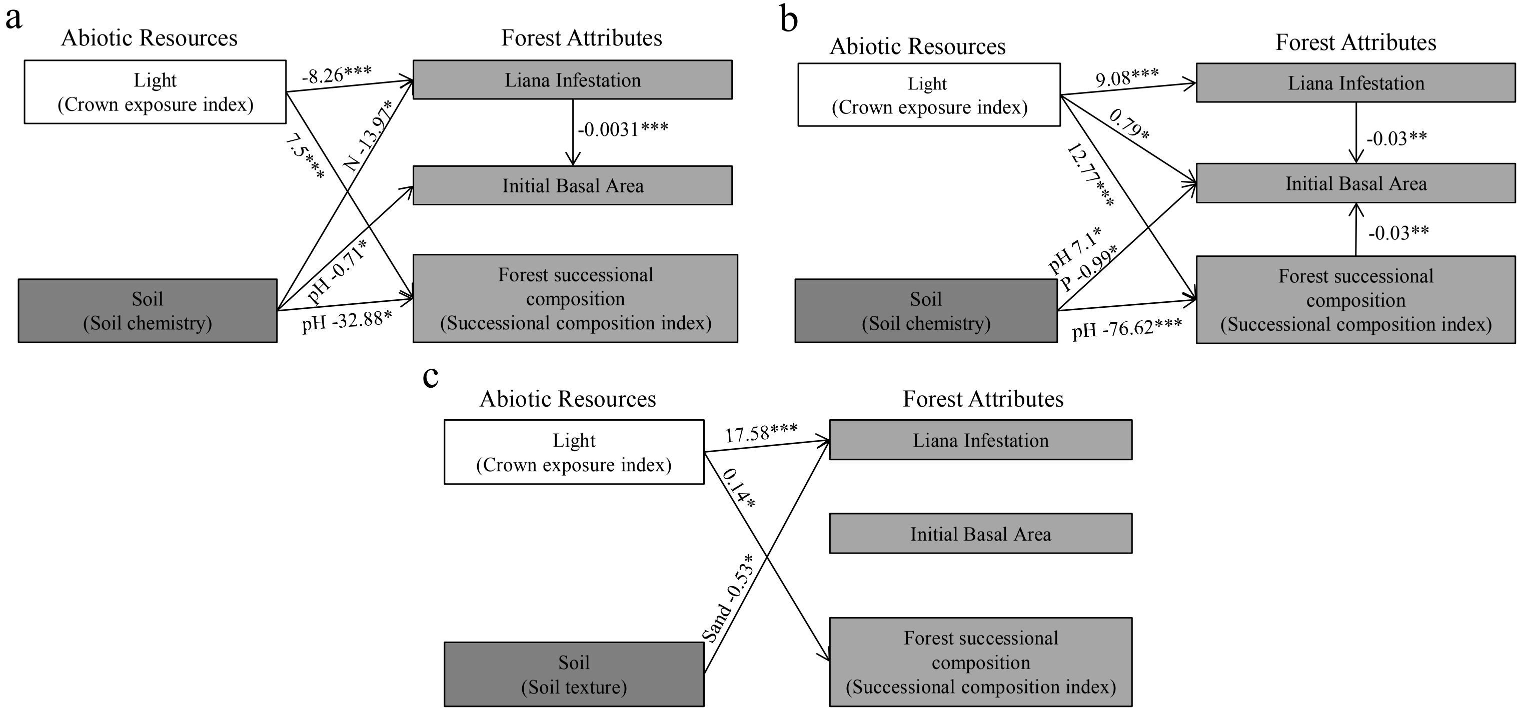


Figure 3.2 Results of the effects of abiotic resources (soils and light) on forest attributes for three size categories of trees **a)** large trees > 40 cm in DBH; **b)** intermediate-sized trees 20 - 40 cm in DBH; **c)** small trees 10 - 20 cm in DBH. Arrows indicate the effect of the explanatory variable on the forest attribute. For each explanatory variable, the absolute regression coefficient and significance level are given (ns= not-significant, * < 0.05, ** < 0.01, *** < 0.001). For small trees, none of the soil chemical variables was important but soil texture (sand) was selected as predictors in the models.

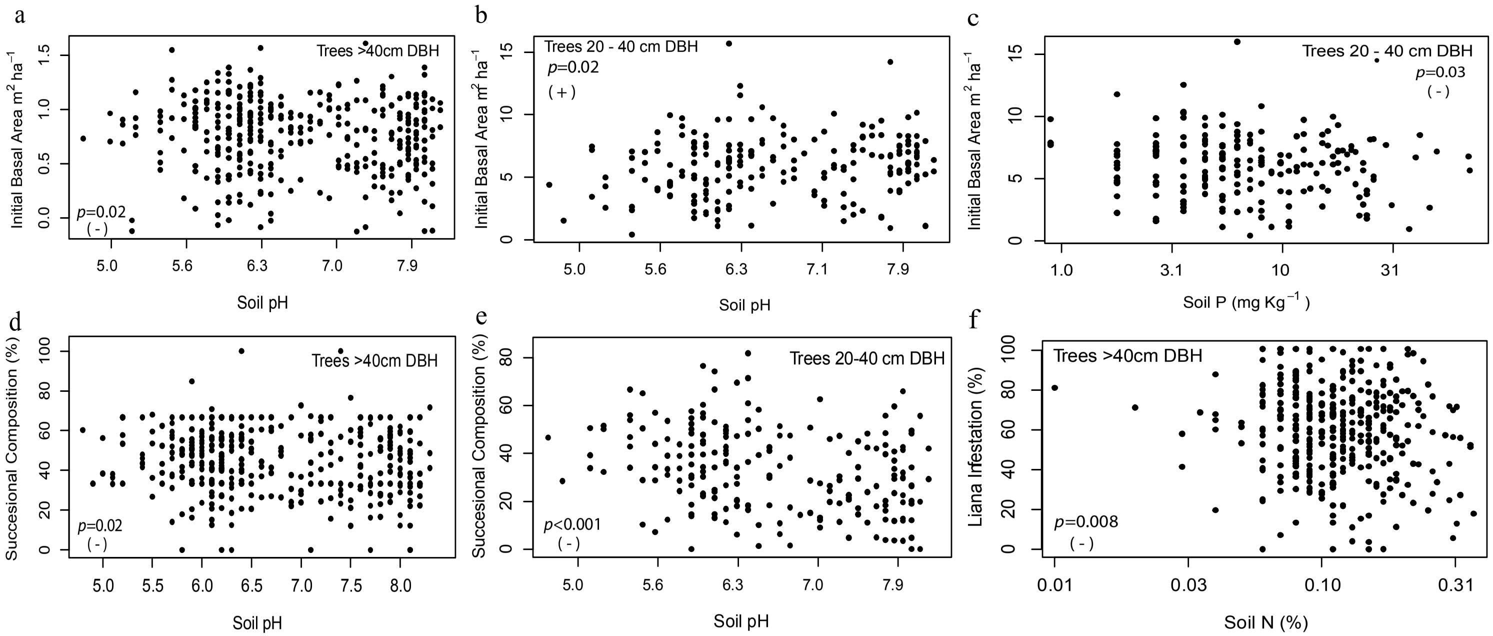


Figure 3.3 Relation between forest attributes: initial subplot basal area; liana infestation: from 0 (subplots where stand biomass has not liana infestation) to 100 % (subplots where stand biomass is completely covered with lianas); and successional composition from 0 (subplots with only shade tolerant trees) to 100% (subplots with only short-live pioneer trees) and soil resources (pH, P and N) for the tree size classes where the relationships were significant. N = 377 for trees > 40 cm DBH (a, d, f) and N = 205 for trees between 20 and 40 cm DBH (b, c, e). Regression coefficients for final modes are in Table 3.2. The *p* values and the direction of the relationship are shown by the sign between brackets. Please note that the x-axes are $\log_{10}$ transformed (Table A3.1).

Table 3.2 Results of the linear mixed model selected for each of the forest attributes (initial basal area, liana infestation, and successional composition) on each tree size category. For every explanatory variable, the value of the regression coefficient with significance level (*p*) are presented. AIC scores for the 'best' model (Appendix, Table A3.1) that had significant regression coefficients for the explanatory variables are presented as well as the squared Pearson correlation between the fitted model and the empirical data for each model. We also present the value of the model intercept with its significance level (*p*).

Forest Attributes (Response Variable)	**Tree Size Category**	**Explanatory Variables**	**Coefficient**	***p***	**Model AIC**	**Model pseudo-r^2**	**Intercept**	***p***
Initial Basal Area	DBH > 40 cm	Soil pH	-0.71	0.02	227.15	0.12	1.52	<0.001
		Liana Infestation	-0.0031	<0.001				
	20-40 cm in DBH	Soil pH	7.10	0.025	949.84	0.14	1.05	0.69
		Soil P	-1.00	0.035				
		Liana Infestation	-0.03	0.006				
		Succession Composition	-0.03	0.005				
		Crown Exposure	0.79	0.033				
	10-20 cm in DBH	Clay	-0.02	**0.097**	122.53	0.36	0.54	0.005
Liana Infestation	DBH > 40 cm	Soil N	-13.97	0.011	3390.11	0.14	82.10	<0.001
		Crown Exposure	-8.26	0.001				
	20-40 cm in DBH	Crown Exposure	9.08	<0.001	1767.97	0.17	21.65	0.014
	10-20 cm in DBH	Crown Exposure	17.58	<0.001	804.01	0.33	33.06	0.02
		Sand	-0.53	0.015				
Succession Composition	DBH > 40 cm	Soil pH	-32.88	0.022	3157.29	0.06	39.13	0.008
		Crown Exposure	7.50	<0.001				
	20-40 cm in DBH	Soil pH	-76.62	<0.001	1720.34	0.24	53.55	0.001
		Crown Exposure	12.77	<0.001				
	10-20 cm in DBH	Crown Exposure	0.14	0.04	114.63	0.04	0.93	0.00

Effects of soil, light and forest attributes on forest dynamics

From the components of forest dynamics, basal area growth of intermediate-size trees was highest (0.14 ± 0.08 $m^2ha^{-1}y^{-1}$), lowest for large trees (0.074 ± 0.07 $m^2ha^{-1}y^{-1}$) and intermediate for small trees (0.10 ± 0.09 $m^2ha^{-1}y^{-1}$). Basal area recruitment was rather even for all three size classes, just a bit higher in large and intermediate-size trees (large-size: 0.19 ± 0.15 $m^2ha^{-1}y^{-1}$; intermediate-size: 0.18 ± 0.12 $m^2ha^{-1}y^{-1}$; small-size: 0.14 ± 0.13 $m^2ha^{-1}y^{-1}$). Basal area mortality was highest for large trees (0.27 ± 0.26 $m^2ha^{-1}y^{-1}$), lowest for small trees (0.09 ± 0.06 $m^2ha^{-1}y^{-1}$) and intermediate for intermediate-size trees (0.18 ± 0.14), across a time window of 8 to 10 years.

Soil variables had a weak influence on forest dynamics. Some of the ‘best’ models that explained forest dynamics, selected pH, N, P, OM content and texture to explain basal area growth, basal area recruitment and basal area mortality (Appendix). Nevertheless, only in few cases soil variables were retained as significant in the final model (Table 3.3; Fig. 3.4 a, b, c). Possibly the small size of our plots (radius= 25 m *ca*. 0.2 ha) prevented picking-up stronger patterns due to small amount of trees in the sampled area. In general, basal area growth of survivors was not affected by soil variables, it was only affected by light and forest attributes. For large trees successional composition index negatively affected basal area growth and for intermediate-size trees, successional composition index positively affected basal area growth (Table 3.3), which means that when trees are large, then plots that contain less ‘pioneers’ grow faster, but when trees are of intermediate-size, then plots that contain more ‘pioneers’ grow faster. Only in the case of intermediate-size and small trees, light had a positive effect on basal area growth (Fig. 3.4b, c; Table 3.3); and for intermediate-size and large trees liana had a negative effect on basal area growth (Fig. 3.4a, c; Table 3.3).

Soil variables had only an effect on basal area recruitment of intermediate-size trees. Soil pH had a positive effect on basal area recruitment whereas N and clay had a negative effect (Fig.3.4b; Fig. 3.5a, b, c). Light had a positive effect on basal area recruitment for intermediate-size and for small trees (Fig. 3.4b, c). Among the forest attributes, successional composition had a positive effect on basal area recruitment of small trees and liana infestation did not have any effect (Table 3.3; Fig. 3.4c). Basal area mortality was only affected by soil variables in the case of large trees. Soil pH had a negative effect on basal area mortality (Fig. 3.4 a; Fig. 3.5d; Table 3.3), and the presence of ceramics in the soil had a negative coefficient related to basal area mortality of small trees (Fig. 3.4c; Table 3.3). Basal area mortality was positively affected by initial basal area for all categories (Fig. 3.4a, b, c); positively affected by successional composition in the case of intermediate-size and small trees (Fig. 3.4b, c); and not affected by liana infestation. Lastly, presence of ceramics, included as explanatory variable in the models, was sometimes selected in the ‘best’ model (Annex 3, Table A3.1) but was only significant in explaining basal area mortality of small trees (Fig. 3.4c, Table 3.3).

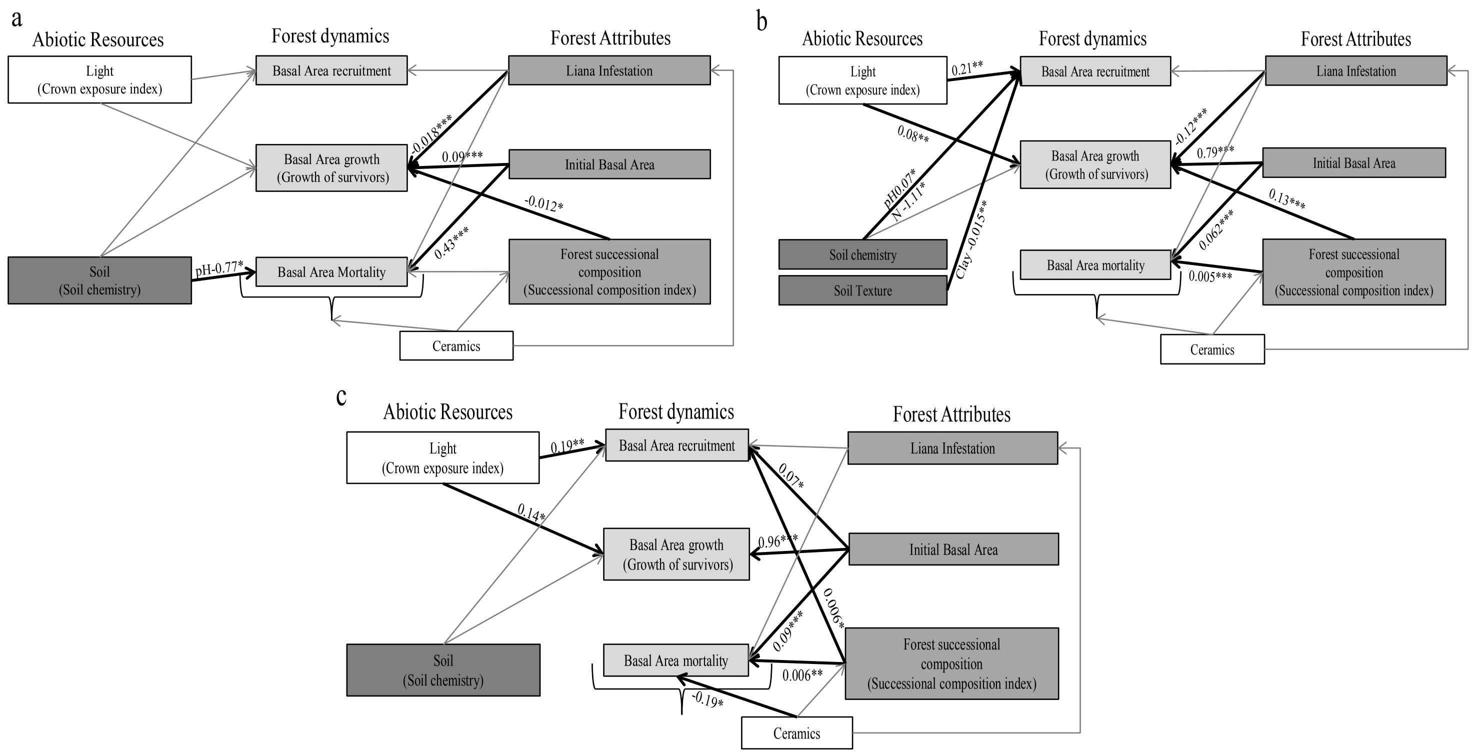


Figure 3.4 Effects of abiotic resources (soil chemistry, soil texture and light) and forest attributes (initial basal area, liana infestation and successional composition) on forest dynamics measured as basal area recruitment, basal area growth and basal area mortality (dead trees) for **a)** large trees > 40 cm of DBH; **b)** intermediate-sized trees between 20 and 40 cm DBH; and **c)** small trees between 10 and 20 cm DBH. Arrows indicate the effect of the explanatory variable on the forest dynamic variable and dashed grey arrows represent relationships that we hypothesised (Fig. 1), but that were not supported by the models. Regression coefficient and significance level are presented (ns = not significant, * < 0.05, ** < 0.01, *** < 0.001).

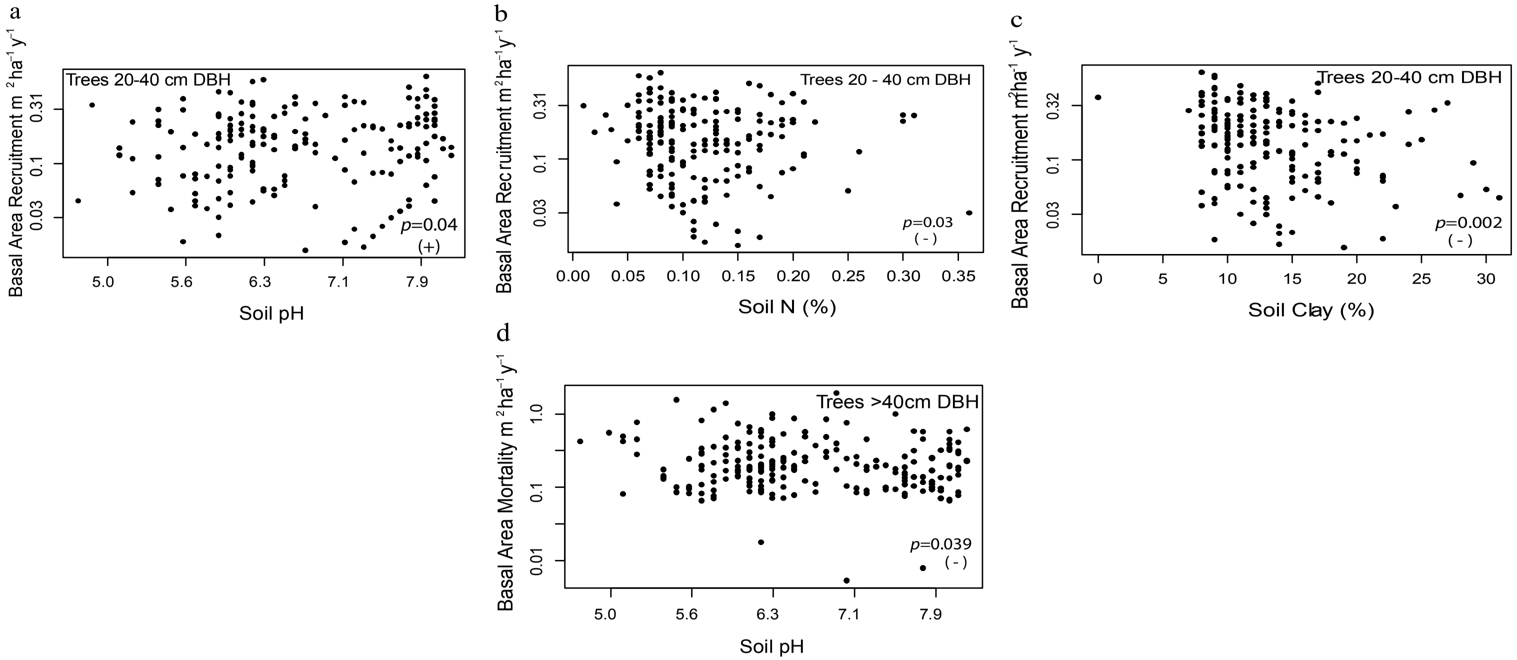


Figure 3. 5 Bivariate scatter plots of the relationship between abiotic resources soil chemistry and soil texture and basal area recruitment (panels a through c) and mortality (panel d). Panels a), b), and c) represent the effect of soil chemistry (pH, N) and texture (clay) on basal area gain; and panel d) shows the effect of soil chemistry (pH) on basal area loss. For the tree size classes where the relationships were significant. Regression coefficients are in Table 3.3. The *p* values and the direction of the relationship are shown by the sign between brackets. Please note that the x-axes are $\log_{10}$ transformed (Table A3.1).

Table 3.3 Results of the linear mixed model selected for each of the forest dynamics variables (basal area growth, basal area recruitment, and basal area mortality) on each tree size category. For every explanatory variable, the value of the regression coefficient with significance level (*p*) are presented. Akaike's Information Criterion (AIC) scores for the 'best' model (Appendix, Table A3.1) that had significant regression coefficients for the explanatory variables are presented as well as the squared Pearson correlation between the fitted model and the empirical data for each model. We also present the value of the model intercept with its significance level (*p*). Note that there is only a model in which the explanatory variable does not have a statistically significant regression coefficient (showed in bold).

Forest Dynamics (Response Variable)	**Tree Size Category**	**Explanatory Variable**	**Coefficient**	***p***	**Model AIC**	**Model pseudo-r^2**	**Intercept**	***p***
Basal Area Growth	DBH > 40 cm	Initial Basal Area	0.10	<0.001	-899.34	0.33	0.06	<0.001
		Liana Infestation	-0.02	<0.001				
		Succession Composition	-0.01	0.039				
	20-40 cm in DBH	Initial Basal Area	0.79	<0.001	-7.04	0.58	-1.63	<0.001
		Liana Infestation	-0.003	<0.001				
		Succession Composition	0.004	<0.001				
		Crown Exposure	0.08	0.005				
	10-20 cm in DBH	Initial Basal Area	0.96	<0.001	30.16	0.54	-1.72	<0.001
		Crown Exposure	0.14	0.034				
Basal Area Recruitment	DBH > 40 cm	Initial Basal Area	0.15	**0.082**	82.87	0.03	-0.92	<0.001
	20-40 cm in DBH	Soil pH	0.07	0.014	132.51	0.23	-1.77	0.007
		Clay	-0.02	0.002				
		Soil N	-1.11	0.032				
		Crown Exposure	0.21	0.001				
	10-20 cm in DBH	Initial Basal Area	0.07	0.015	91.42	0.32	-1.78	<0.001
		Crown Exposure	0.20	0.004				
		Succession Composition	0.006	0.021				

Table 3.3 (continuation)

Forest Dynamics (Response Variable) (Continuation)	**Tree Size Category**	**Explanatory Variable**	**Coefficient**	***p***	**Model AIC**	**Model pseudo-r^2**	**Intercept**	***p***
Basal Area Mortality	DBH > 40 cm	Initial Basal Area	0.43	<0.001	132.51	0.19	0.45	0.15
		soil pH	-0.77	0.039				
	20-40 cm in DBH	Initial Basal Area	0.06	0.015	81.00	0.37	-1.43	<0.001
		Succession Composition	0.005	0.006				
	10-20 cm in DBH	Initial Basal Area	0.08	<0.001	54.06	0.39	-1.53	<0.001
		Succession Composition	0.006	0.005				
		Ceramics	-0.19	0.037				

Discussion

What factors affects forest attributes on this forest?

The effect of soils in forest attributes

We predicted that higher nutrient concentrations associated with ADE would lead to faster growth and hence, a larger initial basal area and a larger infestation by lianas as they prefer higher amount of soil resources. We did not find support for our hypothesis, probably because the relationships between forest attributes and soils were more complex than expected. In our study, soil pH had a positive effect on initial basal area of intermediate-sized trees but a negative effect on that for large trees (Fig. 3.2a, b). Soil pH seems to be a good indicator of overall soil fertility in la La Chonta (Pearson correlations for N: 0.52; for P: 0.34; for K: 0.49; for Ca: 0.74). Nevertheless, in our forest initial basal area of intermediate-size trees was negatively related with P (Fig. 3.2b). This low productivity at increased soil P contrasts with the idea that P is the main limiting nutrient of forest productivity (Vitousek et al. 2010) as well as with findings from lowland tropical forests in Borneo (Paoli and Curran 2007, Paoli et al. 2008) and across the Amazonian forest (Aragão et al. 2009, Quesada et al. 2012), where soil P significantly increased forest biomass. It is in agreement, however, with other studies from lowland Bolivia that show negative relationship between stem density and soil nutrients (Toledo et al. 2011b). One possible explanation for this contradiction may be the effects of soil pH on plant nutrition. For instance, an increase in Ca related to ADE may change soil pH, which in turn may limit plant nutrition, by decreasing the availability of some minerals such as Fe, Zn, Mn, and P (Lambers et al. 2008b). Unfortunately we do not have data on soil availability of Fe, Zn and Mn to confirm this. We have evidence that an increase in P does not translate into an increase in plant growth, as it actually changes nutritional stoichiometry. This evidence comes from a greenhouse experiment, where we compared seedling growth of 17 species on ADE and non-ADE soil from the same site (Chapter 4 this thesis). The response of large trees seemed to be in line with this argument because initial basal area and soil pH were negatively correlated. This physiological constraint related to soil pH may as well also influence the successional composition of the plots. Our successional composition index was negatively related with soil pH, indicating that plots with lower pH have more long-lived pioneer species whereas plots with high pH have more shade tolerant species. Pioneer species have more acquisitive strategies than shade tolerants and need higher availability of nutrients, which may not be available at higher soil pH. A previous study showed that a gradient in soil pH in La Chonta affected understory species composition (Quintero-Vallejo et al. 2015). Soil pH also was shown to affect tree species composition in a lowland tropical forest in Panama (Jones et al. 2013) and '*cerrado*' vegetation in south-east Brazil (Viani et al. 2014). We suggest that an interplay among soil pH, nutrient availability and changes in forest composition could contribute to disentangle the complex patterns found here, but recognize that further research is needed.

We hypothesized that nutrients on ADE could positively affect liana infestation. We found a rather negative effect of soil N in liana infestation of large trees. We do not have a clear explanation for this finding, since when lianas show association with soil fertility, it is rather positive (DeWalt and Chave 2004), and it is expected that, as fast growing species, lianas perform better in richer soils (Kazda 2015).

The effect of light on forest attributes

We hypothesized that light should have a positive effect on the dominance of inherently light-demanding species, such as (long-lived) pioneer trees and lianas. We confirmed this hypothesis since light was positively related to successional index for all size categories indicating that the more illuminated places had higher proportion of long-lived pioneers. Light also was positively correlated to liana infestation of intermediate and small trees although it negatively affected liana infestation of large trees. This last results was unexpected. Possibly large canopy trees that received much light successfully overcome lianas, whereas those with high liana infestation remain more suppressed in the canopy. The effect of light on liana infestation was stronger for small trees suggesting that in the understory or sub-canopy of the forest, lianas might be limited by light and could be more responsive to openings of forest gaps (Schnitzer et al. 2000, Dalling et al. 2012). Liana infestation had a negative effect on the initial basal area of large and intermediate-size trees, reflecting their negative effect on tree growth and the implication thereof in tree mortality (Phillips et al. 2005, Peña-Claros et al. 2008).

In conclusion, forest attributes are strongly influenced by light and much less by ADE soil characteristics. Although soils at La Chonta are heterogeneous, as a consequence of geological history of the site, nutrient levels are in the range of high fertility with P, Ca, K and N levels similar to those in south-west Amazonian region (Quesada et al. 2010). Under such conditions plants may not be limited by nutrients, and consequently, a human related increase in soil nutrients will not have a substantial effect in forest productivity.

What affects forest dynamics on this forest?

The effect of soil on forest dynamics

We predicted that high soil nutrient concentrations associated with ADE would affect forest dynamics through a positive effect on basal area growth and recruitment, but that faster growth should also lead to a higher basal area mortality, and together to an increase in the overall forest dynamics. The results do not support our hypothesis since we found that soils did not have a strong effect on forest dynamics and when there was an effect, it was negative. Soil N was negatively related to basal area recruitment of intermediate-size trees (Fig. 3.4b). This negative relationship between forest dynamics and soil nutrients has been found earlier for the lowlands of Bolivia (Toledo et al. 2011b). The role of N in forest dynamics is debated since recent studies indicated that N did not significantly affected tree growth in lowland tropical forest in Panama (Wright et al. 2011) nor in Costa Rica (Baribault et al. 2012). Our negative relationship between N and basal area recruitment may be associated with the complex interactions between soil pH, nutrient availability and species composition discussed above (Chapter 4; Quintero-Vallejo et al. 2015). Interestingly, intermediate-size trees were the tree category more responsive to soils; this has been suggested for other tropical forest (DeWalt et al. 2006, Paoli and Curran 2007). Possibly small trees are more limited by light than by soil, while large trees may have entered a senescent stage unrelated to soil conditions. Here, large trees had lower basal area mortality with increasing soil pH, possibly due to effects of soil pH on species composition and basal area: sites with higher pH had a higher proportion of shade tolerant species with inherently lower mortality rate (Poorter et al. 2008).

In contrast to our prediction, none of the soil variables affected basal area growth in any of the tree size categories (Fig. 3.4). This result is in contradiction with several studies from the Amazonian region and other tropical forests where increases in soil nutrients, especially soil P leads to an increase in forest biomass (Quesada et al. 2012) and net primary productivity (Paoli and Curran 2007, Paoli et al. 2008, Aragão et al. 2009). In fact, studies that evaluated 1-ha forest in sites of ADE in central Amazonian forest, which is characterised by a very low fertility of underlying soil, found that soil fertility of ADE, especially increases in soil P, had a positive effect on net primary productivity, mainly caused by an increase in belowground biomass (Aragão et al. 2009, Doughty et al. 2014).

Delimiting the boundaries of ADE patches in La Chonta forest based on soil color is challenging given the overall dark color of the natural soils. In our preliminary soil surveys we added records on presence of ceramics, clearly indicating the presence of past human inhabitation in the sites. In all models we included presence of ceramics as explanatory variable, but rarely it was selected in the ‘best’ model (Appendix, Table A3.1). Ceramics was only retained as explanatory variable in one model, and was only weakly significant in explaining basal area mortality of small trees (Fig. 3.4c). We do not have an explanation for this result since we did not find significant correlations between ceramics and any of the soil variable in the whole soil database. This could be related to not very accurate ceramics sampling that could show the extension of the human occupation.

The effect of light and forest attributes on forest dynamics

Basal area growth was more affected by light and forest attributes (Fig. 3.4) than by soil. Light promoted the growth of small and intermediate-size trees occupying the middle strata of the forest, but it did not have any effect on large canopy trees, possibly because all large trees are already well-exposed (average CI = 4.4). Liana infestation, however, affected growth of intermediate-size and large trees but not small trees; maybe because small trees are used by lianas only as support on their way up to the forest canopy. Therefore, liana effects in the shaded understory may be less strong. Finally, successional composition of the plots had, surprisingly, a negative effect on the growth of large trees (i.e. plots with more pioneers have slower growth), while it was positively related to growth of intermediate-size trees (i.e. plots with more pioneers have faster growth), which is in line with the expectations. The large pioneer trees may be reaching their maximum age/size, when growth rate tends to slow down, while the more long-lived shade tolerant larger trees keep on growing in diameter. Plots with many pioneer of intermediate-size trees are probably also more open and as pioneers can take better advantage of this light, the overall growth is higher than in plots with more shade tolerant intermediate-size trees.

The contribution of tree sizes to the whole-forest dynamics is different. Large trees have a low net contribution, due to the combination of high mortality and low growth. Intermediate-size trees mainly contribute to forest dynamics through their basal area growth (0.14 ± 0.08 m^2 ha^{-1} y^{-1}) since basal area recruitment and basal area mortality are the same. Finally, small trees contribute to forest dynamics by having higher basal area of recruits compared to basal area growth and basal area of dead trees. Hence, for the overall-forest dynamics, those factors that contribute to the growth of the intermediate-size trees and recruitment of small trees are crucial. Our results indicate that the growth of small trees is determined by standing basal area, light, and successional composition and that the growth of intermediate-size trees is determined by standing basal area, light, successional composition and liana infestation. Thus, the effect of soil variables in the dynamics of La Chonta forest is mediated through the soil effects on standing basal area and successional composition of intermediate-size and small size trees.

Conclusions

In our study site, which is located at the higher end of the soil fertility gradient observed across the Amazonian region, ADE extends soil fertility by increasing soil P, soil Ca, and soil pH. But, this increased in soil fertility does not directly enhance forest growth. Our results suggest that soils and light drive forest attributes at La Chonta. Light will affect the successional composition of the forest and the level of liana infestation of trees. Soils will affect the standing basal area of large and intermediate-size trees, as well as the successional composition of the plots. The specific effects of soil nutrients on forest dynamics are negative, rather than positive and are mediated through the effect of soil on forest attributes rather than directly.

Acknowledgements

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Appendix.

Laboratory procedures for obtaining soil chemical and physical variables

The concentrations of N, P, exchangeable Ca^{2+}, Mg^{2+}, K^{+} , Al^{+3}, pH, organic matter content (OM), total exchangeable bases (TEB), cation exchange capacity (CEC), base saturation, acidity, and soil texture were analysed. Soil texture (percentage of sand, silt, and clay) was measured using a Bouyoucos hydrometer; pH and electric conductivity were determined in 1:5 distilled water equilibrating for 90 min. Concentrations of exchangeable Ca^{2+}, Mg^{2+}, K^{+} and Na^{+} were determined in 1 M ammonium acetate at pH 7. After extraction, Ca^{2+} and Mg^{2+} were measured with an Atomic Absorption Spectrophotometer; concentrations of K^{+} and Na^{+} were measured with a flame photometer; exchangeable aluminium (Al^{3+}) was measured in 1 M hydrochloric acid (HCl). Total P was determined by Olsen method; OM was determined by Walkley-Black method, and total N was measured using the Micro-Kjeldahl method (Barber and Cochrane 1993). TEB was defined as the sum of the most important exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}). CEC was calculated as the sum of TEB plus acidity. Base saturation was computed as the percentage of exchangeable cations or bases relative to total CEC.

Table A3.1 Result of the best model after model selection. Models presented here correspond to the best selected model with the lowest AIC score. We also present the *Akaike weight* (*Akaike w*). *Akaike w* is referred as the 'weight' of evidence in favour of the model selected as being the 'best' model for the data compared with a group of possible models. The closer to 1 the better predictive power the 'best' model have (Burnham and Anderson 2012). Note that variables are presented in the scale that they were evaluated (log transformed or without transformation). Bolded explanatory variables Shown in bold where the variables that were retained for the final model after model averaging and selection based on their importance value (Barton 2012) and whose regression coefficient was statistically significant (Table 2 and Table 3).

	Response Variable	**Tree Size Category**	**Model**	**Random F**	***Akaike w***
Forest Attributes	Initial Basal area	Trees bigger than 40 cm	logInitialInitial BA= **logpH + liana infestation** + clay + Ceramics + logpH*Ceramics	Plot, Block	0.029
		Trees between 20 and 40 cm	Initial BA= **Succession composition + logpH +logP + liana infestation + Crown Exposure**	Plot, Block	0.058
		Trees between 10 and 20 cm	logInitial BA= **clay**	Plot, Block	0.021
	Liana Infestation	Trees bigger than 40 cm	Liana Infestation = **logN + Crown Exposure** + Succession Composition	Plot, Block	0.046
		Trees between 20 and 40 cm	Liana Infestation= **Crown Exposure** + Clay	Plot, Block	0.034
		Trees between 10 and 20 cm	Liana Infestation= **Crown Exposure + Sand** + logOM + logpH	Plot, Block	0.081
	Succession Composition	Trees bigger than 40 cm	Succession Composition = **logpH + Crown Exposure**	Plot, Block	0.064
		Trees between 20 and 40 cm	Succession Composition = **logpH +Crown Exposure** + Sand	Plot, Block	0.06
		Trees between 10 and 20 cm	Succession Composition = **Crown Exposure** + Clay	Plot, Block	0.021
Forest Dynamics	Basal Area Growth	Trees bigger than 40 cm	Basal Area Growth = **logInitial BA + Liana Infestation + Succession Composition** + Ceramics	Plot, Block	0.024
		Trees between 20 and 40 cm	Log Basal Area Growth = **logInitial BA + Liana Infestation +Succession Composition + Crown Exposure** + logpH + logOM + logP + Ceramics + logpH*Ceramics	Plot, Block	0.037
		Trees between 10 and 20 cm	Log Basal Area Growth = **logInitial BA + Crown Exposure**	Plot, Block	0.052
	Basal Area Recruitment	Trees bigger than 40 cm	Log Basal Area Recruitment = **logInitial BA**	Plot, Block	0.021
		Trees between 20 and 40 cm	Log Basal Area Recruitment = **logpH+ Clay + Crown Exposure + soil N** + Ceramics*logpH	Plot, Block	0.023
		Trees between 10 and 20 cm	Log Basal Area Recruitment = **logInitial BA + Crown Exposure + Succession Composition**	Plot, Block	0.042
	Basal Area Mortality	Trees bigger than 40 cm	Log Basal Area Mortality = **logInitial BA + logpH**	Plot, Block	0.014
		Trees between 20 and 40 cm	Log Basal Area Mortality = **Initial BA + Succession Composition** + Crown Exposure + logOM + logpH	Plot, Block	0.017
		Trees between 10 and 20 cm	Log Basal Area Mortality = **Initial BA + Succession Composition + Ceramics** + Liana Infestation + logOM	Plot, Block	0.017

Chapter 4

Effects of Amazonian Dark Earths on growth and leaf nutrient balance of tropical tree seedlings

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Abstract

Amazonian Dark Earths (ADE) are ancient anthropogenic soils distributed in the Amazon basin. They are characterized by high nutrients concentrations, such as those of phosphorus, calcium, potassium and nitrogen and increases in soil pH when compared with natural soils. We studied the effect of ADE on seedling growth, morphology and physiology in a greenhouse experiment with seedlings of 17 tree species from a tropical moist forest, located in the southern boundary of the Amazon basin. Soils were collected in a forest located in the province of Guarayos, Bolivia, where presence of ADE has been reported. Seedlings were grown for 2-4 months on ADE and non-ADE. We evaluated soil nutrient concentration, seedling growth, leaf and root functional traits, and leaf nutrient concentrations. We found that soil type affected 10 out of 24 evaluated seedling traits. Seedlings did not invest more in roots in non-ADE but they invested in leaves and leaf area in ADE, although this did not lead to faster growth rate. Species responded differently to increases in soil calcium in the soil from ADE; some species seemed to suffer from calcium toxicity as is was indicated by seedlings mortality on ADE; others from nutrient imbalance observed from changes in nutrient stoichiometry in the leaves; whereas other species increased their leaf calcium, phosphorus and nitrogen concentrations in ADE. Only for this latter group of nutrient accumulators, there was a positive relationship between leaf calcium and seedlings growth rates. Contrary to our expectations, ADE did not lead to increased seedling growth. Ability of plants to colonize patches of ADE might depend on plant responses to increased soil calcium and their capacity to regulate internal tissue calcium to balance nutrition.

Keywords: Amazonian Dark Earths; Bolivia; Calcium coping strategies; nutrient imbalance; RGR

Introduction

Nutrient availability in tropical soils is the result of complex biogeochemical processes that generate high spatial heterogeneity (Townsend et al. 2008). This heterogeneity is important, since it can determine tree species distribution at different spatial scales (John et al. 2007). However, the exact mechanisms that drive the underlying patterns of plant distribution are still far from being fully understood. This lack of explanation is partly because plants respond differently to a multitude of soil-related factors, such as water (Brenes-Arguedas et al. 2013) and nutrients availability (Ordoñez et al. 2009). Among nutrients, nitrogen (N) and phosphorus (P) are the major limiting elements for primary productivity of forests (Vitousek et al. 2010). Nevertheless, recent fertilization studies have found that potassium (K) and calcium (Ca) influence plant growth in tropical forest in similar ways as N and P (Wright et al. 2011, Baribault et al. 2012).

Plants respond to soil nutrient availability through a suite of traits, by adjusting their allocation patterns, morphology, tissue chemistry and physiology. With an increase in soil nutrient availability, plants shift biomass allocation to aerial structures, such as leaves, instead of underground structures such as roots (Poorter et al. 2012). This is usually accompanied by changes in other plant traits such as an increase in specific leaf area (SLA), N and P concentration in leaves, and growth rates (Ordoñez et al. 2009, Ordoñez et al. 2010). However, in soils with low-nutrient availability, plants grow slowly, have a smaller leaf area and allocate more biomass to roots, thus enhancing nutrient uptake (Baraloto et al. 2006). Plant tissue chemistry changes with soil fertility, which is usually reflected in the nutrient concentration of leaves, particularly leaf N and P and their ratio (Ågren 2004, Townsend et al. 2007). Apart from soil fertility, foliar nutrient concentrations are shaped by a number of other factors, such as soil pH (Lambers et al. 2008a, Viani et al. 2014), plant demands for elements such as calcium (Ca) to increase tissue toughness (Baribault et al. 2012), negative interactions between tissue P and aluminum (Al) when plants tend to accumulate Al (Metali et al. 2015), species phylogeny (Watanabe et al. 2007), and symbiotic relationships between plants and microorganisms (Nasto et al. 2014). As a result, plant nutrient concentrations and their stoichiometry can be uncoupled from those in the soil.

Soil pH is an important factor in plant nutrition; it may limit the availability of a range of elements that are important for plant growth (e.g., iron [Fe] and zinc [Zn]), and may raise the availability of other elements, such as Al, that could cause plant mortality (Lambers et al. 2008a). For example, in soils with a low pH, increases in Al and Fe availability lead to decreases in P availability by reactions of Al and Fe with inorganic P to form plant insoluble compounds, unavailable for plants (McDowell et al. 2003). Furthermore, an increase in soil Al affects root elongation or plant uptake of other elements such as Ca and magnesium (Mg), causing reduced development of roots, depression of growth and potentially plant death (George et al. 2012). In soils with high pH, such as calcareous soils, increased concentrations of available Ca can react with inorganic P producing calcium phosphate, that leaves P unavailable for most plants (Tyler 1996). Besides, in soils with high pH, some elements such as Fe, manganese (Mn), Zn and copper (Cu) precipitate and become unavailable, thus limiting plant growth (Lee 1998).

In general, soils in the tropics have low pH (Von Uexküll and Mutert 1995) leading to a low availability of a range of nutrients. However, Neotropical soils can also be diverse in their chemical properties as a result of different geological processes. In particular, the Amazon basin has experienced a long and dynamic geological history (Hoorn et al. 2010). In combination with different biogeochemical processes (Quesada et al. 2010) this has led to a wide variation in soil characteristics and different availability of nutrients, essentially P and cations such as Ca^{2+}, Mg^{2+}, and K^{+}. Despite the fact that most of the Amazon basin has a low soil fertility, there are patches of Amazonian Dark Earths (ADE) that are the product of past human inhabitation of the region since pre-Columbian times, and have a high soil fertility (Glaser and Birk 2012).

ADE are characterized by a thick dark or gray top layer (until 1.5m deep) with presence of ceramics that indicates past indigenous settlements (Sombroek 1966, Woods and Glaser 2004). ADE have a black color due to high concentrations of organic matter and black carbon, they have high P and Ca, and higher pH than the natural soils commonly found in the Amazon, such as Ultisols and Oxisols (Sombroek 1966, Falcão et al. 2009, Glaser and Birk 2012). ADE seem to be the product of additions of charcoal and organic waste which change soil physic-chemical characteristics (Schmidt et al. 2014). For example, charcoal addition leads to a more stable organic matter content, and a high soil nutrient retention capacity (Glaser et al. 2002), whereas the addition of organic waste leads to high concentrations of P, N, and Ca (Glaser 2007). Compared with natural Central Amazonian soils, ADE are more fertile because of the combination of high pH and Ca and P concentrations. For this reason, ADE are often used by local farmers to grow crops faster for longer periods of time compared with slash and burn agriculture done on regular soils in the Amazon (German 2003). Similarly, a forest growing on ADE increased gross primary productivity, net primary productivity (NPP), and high rates of fine root production compared with a forest growing on non-ADE (Doughty et al. 2014).

By stimulating productivity, the high fertility of ADE could promote changes in plant traits associated with acquisition strategies, such as induction of a higher relative growth rate, higher leaf biomass and higher nutrients concentration in the leaves. Evaluating changes in plant traits associated to ADE provides a unique opportunity to understand tree responses to soil fertility as a consequence of past human inhabitation and soil modification. Besides, it could help to understand how soil fertility influences species composition in habitats that do not vary in climatic conditions and water availability.

Here we present results of a greenhouse experiment, in which seedlings of 17 tropical tree species were grown on ADE and non-ADE. We examined how soil fertility of ADE affected seedling growth, biomass allocation, and morphological and chemical traits of tropical tree species. We hypothesize that plants growing on ADE have higher growth rates compared with plants growing in non-ADE. Besides, we predict that the higher growth rates, of seedlings on ADE, will be associated with increases in biomass allocation to stem and leaves, leaf N, Ca and P concentrations, specific leaf area and leaf area ratio; traits that enhance light capture and carbon gain. We also predict that seedlings growing on less fertile non-ADE will have higher biomass allocation to (secondary) roots and will produce roots with higher specific root length to enhance nutrient uptake.

Methods

Species and collection site

Seventeen tree species were selected for the study based on seed availability. Hereafter species will be referred by the genus name (Table 4.1). Most of the seeds were collected from a semi-evergreen forest (La Chonta forest concession) located in the province of Guarayos, Bolivia (15º47'S, 62º55'W) in July and August of 2012. The site has a mean annual temperature of 24.3°C and mean annual rainfall of 1580 mm (data from 2000-2006 from La Chonta) with a dry season (<100 mm/mo) from May through September (Peña-Claros et al. 2012). Soils at La Chonta are a mosaic of poor soils from the Brazilian Precambrian Shield (Navarro and Maldonado 2002) and more fertile soils that are the product of sedimentation and erosion originated from the uplift of central Andes (Latrubesse et al. 2010). A previous study showed that La Chonta also has Amazonian Dark Earths (ADE); anthropogenic soils that imply inhabitation of this forest in the past (Paz-Rivera and Putz 2009).

Study site

The experiment was carried out in a shade house at the Agronomy Faculty of the Universidad Autónoma Gabriel René Moreno in Santa Cruz de la Sierra, Bolivia (16º30'S, 63º10'W). The climate in this city is warm, with an average temperature of 24.2 ± 0.8 °C, and mean annual rainfall of 1308 ± 391 mm (Data from National Institute of Meteorology, Bolivia; 1949-2013). The 6 x 3 m shade-house was built and covered with black mesh (2 x 1.5 mm) to give an irradiance of about 10% of full sunlight (around 400 lux).

Soils and shade-house experiment

The soil used for testing germination and plant growth was collected at La Chonta, where we previously identified a site with dark earth and the presence of pottery shards, hereafter ADE, and a site at least 500 m away with more clear soils, hereafter non-ADE. Soils were mixed with river sand at a ratio of 4:1 (w/w), to allow adequate drainage and to facilitate harvesting of the whole root system, including fine roots. Although the use of sand could dilute the effect of soil nutrients, we considered that this dilution might not affect our experiment because the differences between ADE and non-ADE were retained for most of the soil variables, except for total N (Appendix 4, Table A4.1). Chemical and physical characteristics of the original soils and of the mixture with sand were assessed in the Centro de Investigación Agrícola Tropical (CIAT), in Santa Cruz, Bolivia (protocols for chemical and physical properties can be seen in Online Resource 2). The main differences in soil variables between ADE and non-

ADE sand mixes were found for Ca concentration, P concentration, total exchangeable base and cation exchange capacity (ratios between ADE and non-ADE were > 2 in all cases); soil pH was slightly alkaline (> 7) in both soils (ADE: 7.8; non-ADE:7.6) (Appendix 4, Table A4.1).

Table 4.1 Scientific names and families of tree species used for this study.

Scientific name	Family
Albizia niopiodes (Spruce ex Benth.) Burkart	Fabaceae
Anadenanthera colubrina (Vell.) Brenan	Fabaceae
Enterolobium contortisiliquum (Vell.) Morong	Fabaceae
Erythrina crista-galli L.	Fabaceae
Hymenaea courbaril L.	Fabaceae
Machaerium villosum Vogel	Fabaceae
Ormosia nobilis Tul	Fabaceae
Poeppigia procera C. Presl	Fabaceae
Samanea tubulosa (Benth.) Barneby & J.W. Grimes	Fabaceae
Schizolobium parahyba (Vell.)S.F. Blake	Fabaceae
Terminalia oblonga (Ruiz & Pav.) Steud.	Combretaceae
Cariniana ianeirensis R. Knuth	Lecythidaceae
Chorisia speciosa A. St.-Hil.	Malvaceae
Guazuma ulmifolia Lam.	Malvaceae
Cedrela fissilis Vell.	Meliaceae
Swietenia macrophylla King	Meliaceae
Sapindus saponaria L.	Sapindaceae

From April through September of 2013 seeds were germinated in trays of 25 x 30 cm filled with either ADE or non-ADE sand mixes. When seedlings produced their first leaves, eight seedlings per species per soil type were harvested and 12 seedlings per species per soil type were transplanted to pots of 650 ml (9.2 x 15 cm). The transplanted seedlings were placed in a completely randomized design in the shade-house and were re-distributed at random every three weeks to ensure that all seedlings were growing under similar light and temperature conditions. Plants were watered daily or every other day depending on the weather, since the shade house allowed rain water to pass through. Once seedlings were established, at around half of the growing period, the number of leaves on each seedling was counted and the newest full developed leaves were tagged with colored threads. At the time of harvesting we counted the number of newly produced leaves.

A second harvest was done between 2 to 4 months after transplanting, depending on the species. Differences in length of the growing period were due to differences in germination time, and lack of seed availability of some species at the time we started the experiment. At each harvest, we determined the fresh mass of roots, stems, and leaves; leaves were sectioned into the leaf lamina and the petiole, or rachis for compound leaves, and sections were weighed separately. Laminae were scanned with a desktop scanner (Cannon

LIDE 20, Canon, USA) and leaf area was determined using the software program *imageJ* (Rasband 2008). Leaf resistance to mechanical damage was measured for nine of thirteen species using force to punch. Force to punch was determined on one or two of the newest expanded leaves using a field penetrometer, which consisted of a flat-end nail of 3.2 mm in diameter; the nail was attached to the inner part of a syringe and a water basin on top. A leaf was placed between two acrylic plates both having a 6 mm diameter hole. The holes were located in the same position so that the nail could cross the leaf between the two plates similar to Aranwela et al. (1999). Force to punch the leaf was determined by the weight (converted to Newton) of the water added to the basin that was necessary to penetrate the leaf, divided by the circumference of the punch nail.

Root length (RL) was determined following Newman (1966). Roots were placed in a tray filled with water. The tray was covered by a transparent sheet marked with 1 x 1 cm square grid and the number of intersections between roots and the gridlines were counted horizontally and vertically. Thereafter, total root length was determined as $R = \pi NA/2H$, where R is the total length of the root (cm), N is the number of intersections between the root and the gridlines, A is the area of the rectangle (cm^2) and H is the total length of the straight lines of the grid (cm) (Newman 1966). Root morphology was described by the presence/absence of tap roots and by the number of secondary roots. When tap roots were present both the diameter of the base (close to the transition to stem) and the diameter of the tip were measured. The diameter of three secondary roots was measured with a digital calliper.

We measured stem length from the base to the apical bud, and top and base stem diameter. Stem volume was calculated using the formula for a section of a cone. Lamina and petioles, roots and stems were stored in separate paper bags, oven-dried at 70 °C for 72 h, and weighted again to determine dry weight of plant sections. Using the fresh and dry weights we calculated root mass fraction (RMF, $g\ g^{-1}$), leaf mass fraction (LMF, $g\ g^{-1}$) and stem mass fraction (SMF, $g\ g^{-1}$) as the weight of each plant part over the total seedling dry mass. We also calculated stem, root and leaf dry matter content (SDMC, RDMC, LDMC, respectively, in $g\ g^{-1}$) as the dry mass over the fresh mass of that section. Specific leaf area (SLA, $m^2\ kg^{-1}$) was calculated as the ratio between leaf area and leaf dry mass, leaf area ratio (LAR, $m^2\ kg^{-1}$) as the total leaf area over total seedling dry mass, specific root length (SRL, $m\ kg^{-1}$) as root length over dry root mass and root length per plant mass (RLPM $m\ kg^{-1}$) as the root length over total seedling dry mass.

These traits determine important functional characteristics for the plants. Biomass fractions of seedling sections describe how plants allocate biomass to light intercepting tissue in the case of leaves, or nutrient capturing tissue in the case of roots. Stem, root and leaf dry matter content, and force to punch indicate tissue toughness, which are thought to be good proxies for tissue longevity. SLA and LAR indicate how efficiently plants invest in light interception. Measurements of SRL and PLPM indicate how the biomass that is allocated to roots can be efficient in nutrient capture through increase of absorption surface (Markesteijn and Poorter 2009).

Leaf nutrient N, P, and Ca concentration was determined for three randomly selected seedlings per species per soil type. For 5 species (*Albizia*, *Anadenanthera, Cedrela, Machaerium* and *Samanea*) we selected a seedling that had a leaf dry weight > 50 mg, the minimum quantity required for leaf tissue analyses in the laboratory. Extraction of N, P and Ca were done using digestion with H_2SO_4, Se, and salicylic acid (Novozamsky et al. 1983). After digestion, N and P content was measured with a Skalar San-plus auto analyzer, and Ca content was measured using an Atomic Absorption Spectrometer (AAS) from Varian. Tissue analyses were done at the facilities of Wageningen University, The Netherlands.

Data analyses. We quantified survival percentage for each species dividing the number of seedlings alive at the time of the second harvest by the number of transplanted seedlings. We compared survival percentage between soil types using a chi-square test. Relative height growth rate (RGRh) was calculated for each seedling as: $RGRh = \frac{\ln h_2 - \ln h_1}{t_2 - t_1}$, where h_n represents the seedling height at time n; t_1 is time at transplanting, t_2 time at harvesting, and (t_2 - t_1) is the number of days between transplanting and harvest. RGR based on plant biomass (RGRb) was calculated for each species using the information of both harvests as: $RGRb = \frac{\overline{\ln M_2} - \overline{\ln M_1}}{t_2 - t_1}$, where $\overline{\ln M_n}$ is the average of the natural log of the dry mass of each seedling at harvest n.

Continuous variables were $\log_{10}$-transformed, whereas ratios were arcsine-transformed prior to statistical analyses. To test for the effect of ADE and non-ADE on total biomass, RGRh, and plant traits, we performed a two way ANOVA, with soil type and species as factors. For this analysis we used 13 species instead of 17 because we had significant mortality in 4 species that left us with few replicates per treatment to be able to make comparisons between the two soils. We used an unbalanced design because two species (*Cariniana* and *Hymenaea*) had different number of seedlings per soil type. The interaction between soil type and species was also evaluated. For all traits but RGRh, data of the second harvest were used. The amount of variance that was explained by each factor was determined by dividing the sum of squares associated with each factor (soil or species) and their interaction by the total sum of squares of the model. A separate analysis was done for each species using one way ANOVA, with soil type as factor. The effect of soil type on leaf production rate was tested using a non-parametric Kruskal-Wallis test because the data were not normally distributed.

We detected that the percentage of leaf Ca was always higher on ADE, whereas the percentages of leaf P and leaf N were similar or lower on ADE than in non-ADE, despite the higher concentration of these elements in ADE (see results; Fig. 4.3). Therefore, we tested for the existence of potential relationships between the amount of Ca, P and N acquisition from each soil. We averaged the Ca, N and P concentration in the leaves of seedlings growing in ADE and non-ADE, and calculated the difference between these amounts in each soil type per species, as follows: $Difference_{ADE-nonADE} = (\overline{Nutrient_{i,ADE}} - \overline{Nutrient_{i,non-ADE}})$, where *Nutrient* stands for the concentration in the leaf, and *i* stands for Ca, N or P. Thereafter we tested, using the information of all species, whether the differences in leaf nutrient concentrations between seedlings growing in the two soil types was related between pairs of leaf nutrients performing a linear regression procedure.

Based on the relationships found in the previous analyses (see results; Fig. 4), we separated the species into the following response groups: species in which the difference in leaf Ca concentration was positively associated with the difference in leaf P concentration (hereafter refer as *positive for* P); species in which the difference in leaf Ca concentration was negatively associated with the difference in leaf P concentration (*negative for* P); species in which the difference in leaf Ca concentration was positively associated with the difference in leaf N concentration (*positive for* N); and those species in which the difference in leaf Ca concentration was negatively associated with the difference in leaf N concentration (*negative for* N).

Using the four response groups previously defined, we tested how Ca uptake was related with RGRb. We evaluated Ca because it was the nutrient in the leaves that consistently increased in ADE for all species (Fig. 4.3a). Therefore, we performed a linear regression for each group between leaf Ca concentration in leaves as the independent variable and the average RGRb per species as the dependent variable. Finally, we also tested, using linear regressions, how Ca uptake was related with leaf P uptake or leaf N uptake at the group level. All statistical analyses were done using Genstat 16th ed. (VSN International Ltd).

Results

Eight out of 17 species showed some mortality during the experiment. *Terminalia* and *Poeppigia* had a significantly higher mortality in ADE than in non-ADE soil ($\chi^2 = 10.83$, $p < 0.05$; $\chi^2 = 12.08$, $p < 0.05$, respectively), whereas *Schizolobium* had a significantly higher mortality in non-ADE ($\chi^2 = 15.41$, $p < 0.05$) (Fig. 4.1).

Plant responses to ADE

We found significant differences between soil types for ten out of 24 traits, but the proportion of the variance explained by soils for each of these variables was very low (less than 2.8%, Table 4.2). Species had a significant effect on all variables, explaining 35% to 96% of the variance (Table 4.2).

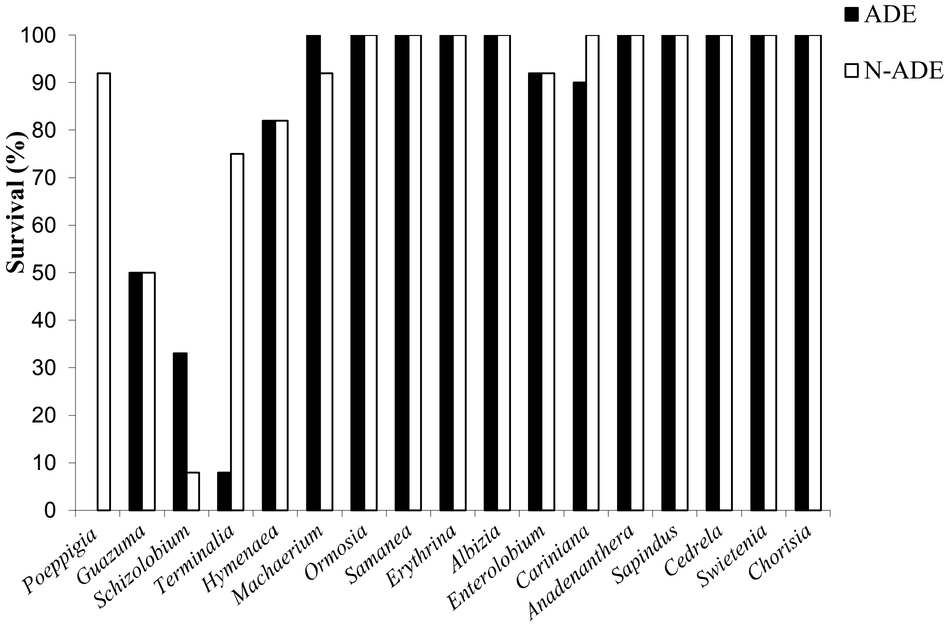

Figure 4.1 Seedling survival of 17 species of tropical trees in Amazonian Dark Earths (ADE) and non-Amazonian Dark Earths (non-ADE) soils after the growing period in the greenhouse.

We expected soil fertility from ADE to have a positive effect on plant growth, but most of the species had a similar growth rate on both soils. Total biomass (Fig. 4.2a), height growth (RGRh, Fig. 4.2b) and biomass growth (RGRb) did not differ significantly between soil types (Table 4.2). However, the interaction between soil type and species was significant for total biomass and RGRh (Table 4.2), indicating that some species grew faster in ADE, whereas other species grew faster in non-ADE. Seedlings of *Cedrela* and *Chorisia* had significantly more biomass in ADE (one-way ANOVA: *Cedrela* F= 7.29, $p = 0.013$; *Chorisia* $F = 5.40$, $p = 0.03$), whereas *Machaerium* had a significantly higher biomass in non-ADE (one-way ANOVA, $F = 4.87$, $p = 0.039$) (Fig. 2a). Furthermore, *Swietenia* had a higher RGRh in ADE (one-way ANOVA, $F = 9.04$, $p = 0.006$) (Fig. 4.2b), whereas *Chorisia* had a significantly higher RGRh in non-ADE (one-way ANOVA, $F = 18.88$, $p < 0.001$).

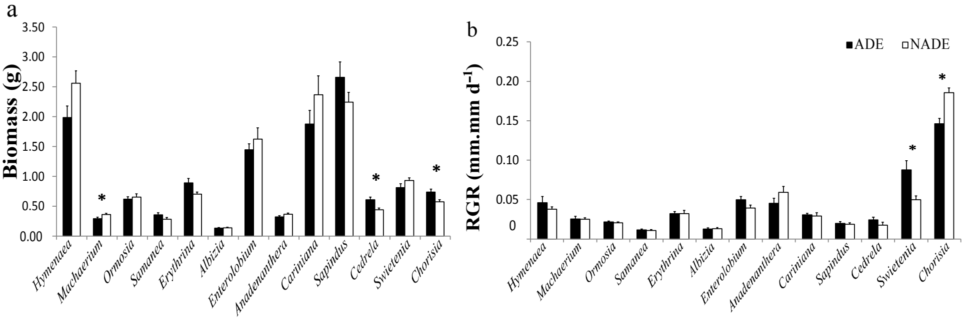

Figure 4.2 Biomass at final harvest (a) and relative height growth rate (RGRh) (b) of seedlings of 13 species growing in Amazonian Dark Earths (ADE) (black bars) and non-Amazonian Dark Earths (open bars). Means and standard errors are shown. Asterisks indicate significant responses of a species to soil ($p \leq 0.05$).

Overall, leaf production rate was similar for both soil types (Kruskal-Walis $H = 2.97$; $p = 0.084$), although some species, such as *Cedrela*, *Chorisia*, and *Cariniana* had a higher leaf production rate in ADE than in non-ADE, whereas *Swietenia* had higher leaf production rate in non-ADE soil (Appendix, Table A4.2). We found that biomass allocation to leaves (LMF) was significantly higher in ADE than in non-ADE (Table 4.2). There were no significant differences in biomass allocation to roots (RMF) between soil types. There was a significant interaction between soils and species in allocation to stems (SMF) (Table 4.2). Seedlings of *Anadenanthera* had heavier stems in ADE ($F = 14.71$, $p < 0.001$) than in non-ADE, whereas *Erythrina* and *Enterolobium* seedlings had heavier stems in non-ADE than in ADE (Appendix, Table A4.2). Specific leaf area (SLA, a measure of area investment per leaf mass) and leaf area investment per plant mass (LAR) were significantly higher for seedlings in ADE (Table 4.2). We found a significant interaction term for specific root length (SRL) (a measure of plant investment in root length) (Table 4.2); *Enterolobium* seedlings had a significantly higher SRL in ADE, whereas *Swietenia* seedlings had higher SRL in non-ADE (Appendix , Table A4.2). Leaf and root dry mass content were significantly higher in non-ADE (Table 4.2). Leaf Ca concentration was significantly higher in ADE (Table 4.2, Fig. 4.3a). Leaf N concentration did not differ significantly between soil types (Table 4. 2, Fig. 4.3b). We found a significant interaction between soil and species for leaf P concentration (Table 2), caused by a significantly higher leaf P concentration in ADE for *Machaerium* and in non-ADE for *Erythrina* (Fig. 4.3c).

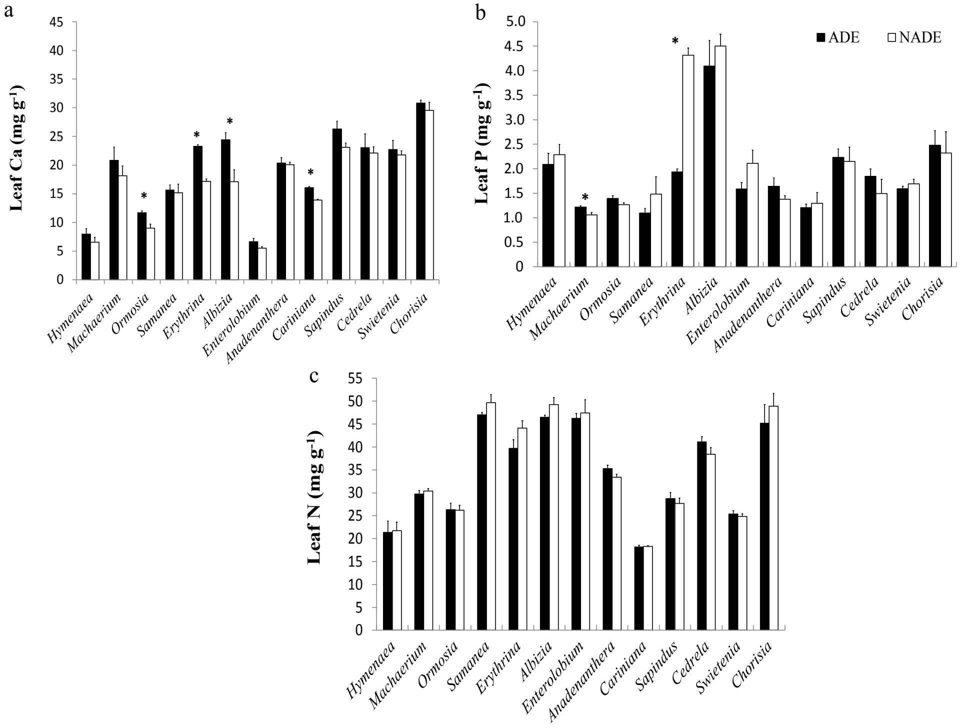

Figure 4.3 Leaf calcium (a), phosphorus (b) and nitrogen (c) concentrations of six seedlings of 13 tropical tree species growing in Amazonian Dark Earths soils (black bars) and non-Amazonian Dark Earths soils (open bars). Twelve seedlings per species were used per each soil type. Bars represent the average per soil type, with standard errors. Asterisks indicate significant differences between the two soils (t-test, $p \leq 0.05$).

Nutrient stoichiometry in relation with growth rates

We explored the differences in leaf nutrient concentration between seedlings growing in ADE and non-ADE soils. We found that all species had a higher leaf Ca in ADE than non-ADE (positive values on *x* axes, Fig. 4a), whereas species had either a higher (positive values on *y* axes, Fig. 4.4a) or a lower leaf P in ADE than in non-ADE (negative values on *y* axes, Fig. 4a). This resulted in a significant negative relationship between the difference in leaf Ca concentration between ADE vs non-ADE and the concomitant difference in leaf P concentration at the species level (Fig. 4.4a). Similar trend was found for leaf Ca concentrations in seedlings growing in ADE vs non-ADE and leaf N concentration (Fig. 4.4); species showed either a higher (positive values on *y* axes, Fig. 4.4b) or a lower leaf N concentration in ADE than in non ADE soils (negative values on *y* axes, Fig. 4.4b). The latter relationship, however, was only a trend, but not statistically significant ($P = 0.086$, Fig. 4.4b). We also found a positive relation between the difference in leaf P and leaf N concentrations in

ADE and non-ADE (Fig. 4.4c), indicating that species that had lower leaf N concentration tended to have lower P concentration on ADE and species that had higher leaf N concentrations also tended to have higher leaf P concentrations on ADE.

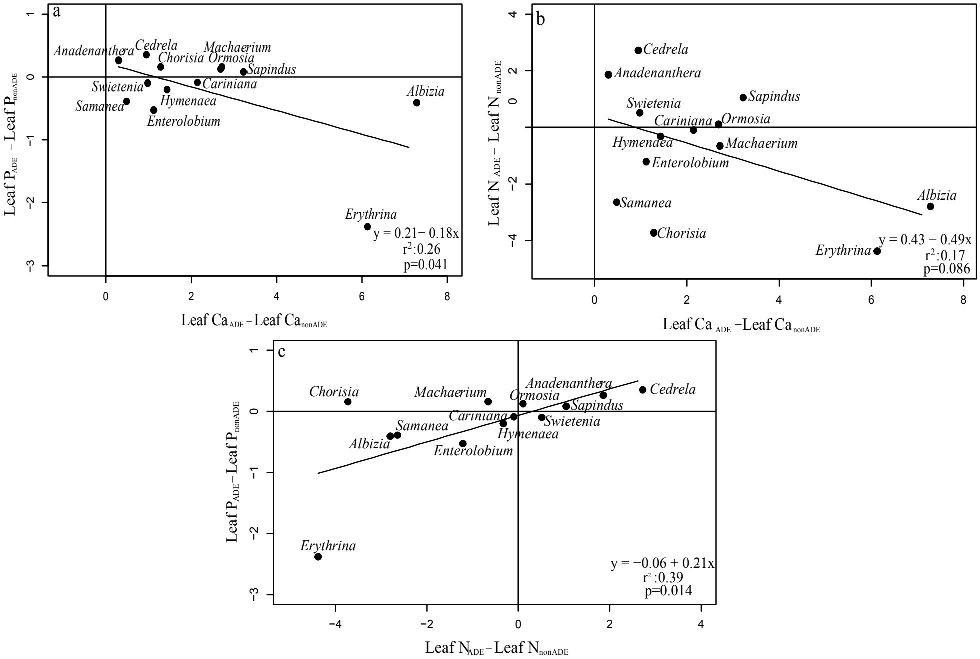

Figure 4.4 Linear regressions of the differences in leaf nutrient concentrations between Amazonian Dark Earths and non-Amazonian Dark Earths. a) Differences in leaf P against differences in leaf Ca. b) Differences in leaf N concentration against differences in leaf Ca concentration; c) Differences in leaf P concentration against differences in leaf N concentration. Results of linear regression are provided. Concentrations units are mg g^{-1}.

To explore the relationship between leaf Ca concentration and either RGRb or leaf P concentration, we divided the species in groups according to their responses to Ca and P or Ca and N. We found that "*positive* for P" species increase in RGRb with increasing leaf Ca concentration (Adj-R^2:0.64; p=0.034) (Fig. 4.5a) and that leaf P concentration increased with leaf Ca concentration, although this was at the edge of significance (Adj-R^2:0.56; p=0.052) (Fig. 4.5b). No relation was found for "*negative* for P" species, neither for RGRb (Adj-R^2:0.09; p=0.26) nor for leaf P concentration (Adj-R^2:-0.04; p=0.42) (Fig. 4.5e, b).

Similarly, we grouped species as "*positive* for N" and "*negative* for N" (Fig. 4.4b) to explore the relationship between leaf Ca concentration and either RGRb or leaf N concentration. We found that "*positive* for N" species significantly increased RGRb with increase in leaf Ca concentration (Adj-R^2: 0.86; p=0.01) (Fig. 4.5c) but they did not increase in leaf N concentration with increasing leaf Ca concentration (Adj-R^2: 0.47; p=0.12) (Fig. 4.5d). On the other hand, "*Negative* for N" species showed no relationship between leaf Ca concentration and either RGRb (Adj-R^2: 0.24; p=0.12) or leaf N concentration (Adj-R^2: -0.05; p=0.46) (Fig. 4.5c, d).

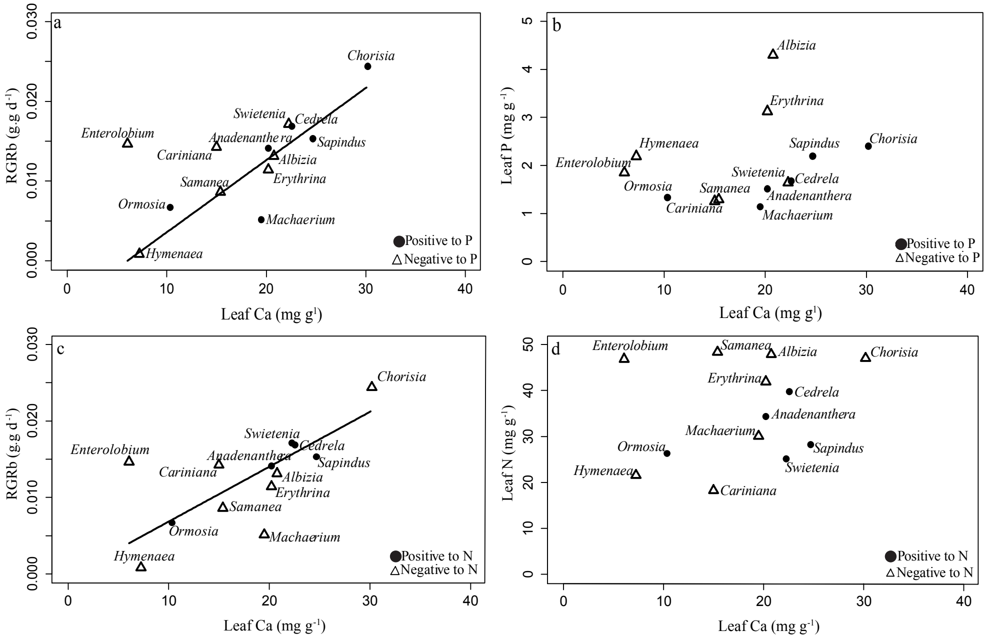

Figure 4.5 Relation between **a**) Relative biomass growth rate (RGRb) and leaf Ca concentration; **b**) Leaf P concentration and leaf Ca concentration for species that responded positively or negatively to P. Lines represent the significant relationship between RGRb and leaf Ca only for species that were "*positive for P*" (RGRb = -0.0054+0.009(leaf Ca); R^2= 0.64; *p*=0.034). Relation between **c**) Relative biomass growth rate (RGRb) and leaf Ca concentration; **d**) Leaf P concentration and leaf Ca concentration for species that responded positively or negatively to N. The regression line represents the significant relationship between RGRb and leaf Ca only for species that were "*positive for N*" (RGRb=-0.0003+0.0071(Leaf Ca); R^2=0.86; *p*=0.014).

Table 4.2. Averages of morphological, physiological and chemical variables measured on seedlings of 13 species of tropical trees growing on ADE (n = 12 seedlings per species) and non-ADE soils (n = 12). The overall R^2 of the model and the ANOVA results are provided. The effect of soil, species or the interaction is presented with their F values and their significance level (*p*). The percentage of variation explained by each factor or the interaction was calculated as the ratio between the factor sum of squares or interaction sum of squares and total sum of squares.

	Average			**Soil**			**Species**			**Soil x Species**		
Trait	**ADE**	**N-ADE**	**R^2**	**F**	***p***	**SSs/SSt**	**F**	***p***	**SSspp/SSt**	**F**	***p***	**SSsxspp/SSt**
Biomas (g)	0.932	0.94	0.89	0.39	0.531	0.0002	184.11	<.001	0.88	**3**	**<.001**	0.014
RGRh (mm.mm.d^{-1})	0.0426	0.0422	0.87	0.09	0.767	0.0000	147.47	<.001	0.84	**6.53**	**<.001**	0.037
RGRb (g.g.d^{-1})	0.01615	0.01595	-	0.005	0.944	0.0000	-	-	-	-	-	-
Root Mass Fraction (RMF) (g g^{-1})	0.232	0.244	0.68	3.36	0.068	0.0040	46.54	<.001	0.67	0.58	0.859	0.008
Stem Mass Fraction (SMF) (g g^{-1})	0.255	0.261	0.67	1.8	0.181	0.0022	43.4	<.001	0.64	**2.15**	**0.015**	0.032
Leaf Mass Fraction (LMF) (g g^{-1})	**0.513**	**0.494**	0.61	**8.06**	**0.005**	0.0117	33.19	<.001	0.58	1.14	0.331	0.020
Diameter secondary roots (mm)	0.481	0.49	0.61	0.08	0.776	0.0001	31.74	<.001	0.59	1.24	0.253	0.023
Secondary to primary root mass (g g^{-1})	0.897	0.755	0.38	2.98	0.086	0.0069	12.57	<.001	0.35	1.05	0.401	0.029
Number of secondary roots	29.17	30.66	0.53	1.43	0.233	0.0026	22.77	<.001	0.49	1.66	0.076	0.036
Specific Root Length (m kg^{-1})	17860	15310	0.57	0.36	0.549	0.0006	26.88	<.001	0.52	**2.69**	**0.002**	0.052
Root Length Ratio (m kg^{-1})	98740	74760	0.78	0.11	0.736	0.0001	79.19	<.001	0.77	1.74	0.059	0.017
Root Dry Matter Content (RDMC) (g g^{-1})	**0.173**	**0.187**	0.78	**15.91**	**<.001**	0.0128	78.82	<.001	0.76	0.78	0.672	0.008
Stem Dry Matter Content (SDMC) (g g^{-1})	**0.282**	**0.293**	0.91	**7.81**	**0.006**	0.0027	218.1	<.001	0.90	**2.12**	**0.016**	0.009
Leaf Dry Matter Content (LDMC) (g g^{-1})	**0.27**	**0.279**	0.96	**14.98**	**<.001**	0.0022	551.38	<.001	0.96	0.98	0.464	0.002
Stem Density (g cm^{-3})	0.31	0.316	0.90	1.6	0.207	0.0006	188.95	<.001	0.89	**1.93**	**0.031**	0.009
Specific Leaf Area (SLA) (m^2 kg^{-1})	**41.66**	**39.46**	0.74	**4.8**	**0.029**	0.0046	63.23	<.001	0.73	0.83	0.624	0.010
Leaf Area Ratio (LAR) (m^2 kg^{-1})	**18.83**	**17.27**	0.61	**9.54**	**0.002**	0.0140	32.52	<.001	0.57	1.17	0.306	0.021
Force to Punch (N mm^{-1})	**0.229**	**0.244**	0.69	**4.31**	**0.039**	0.0065	49.62	<.001	0.68	0.88	0.543	0.012
Leaf Thickness (mm)	0.123	0.124	0.90	1.01	0.315	0.0005	202.28	<.001	0.89	1.87	0.058	0.008
Leaf Ca concentration (mg g^{-1})	**19.2**	**16.8**	0.95	**26.52**	**<.001**	0.0278	71.3	<.001	0.90	1.67	0.102	0.021
Leaf N concentration (mg g^{-1})	34.7	35.4	0.95	1.35	0.251	0.0012	88.88	<.001	0.94	0.87	0.579	0.009
Leaf P concentration (mg g^{-1})	**1.9**	**2.1**	0.90	**6.78**	**0.012**	0.0134	32.42	<.001	0.77	**5.02**	**<.001**	0.119
Ca:P	11.06	9.95	0.83	3.74	0.059	0.0124	18.73	<.001	0.74	1.79	0.075	0.071
Ca:N	**0.59**	**0.51**	0.94	**22.58**	**<.001**	0.0258	65.31	<.001	0.90	1.41	0.191	0.019
N:P	20.51	19.84	0.83	0.41	0.522	0.0013	19.86	<.001	0.77	1.52	0.146	0.059

Discussion

The aim of this study was to evaluate whether tropical tree seedlings respond to the differences in nutrient concentrations between ADE and non-ADE. Seedlings indeed adjusted their morphology and tissue chemistry in response to the increased fertility in ADE but this did not lead to higher seedling growth in ADE. This lack of growth response seems to be determined by the interplay between the uptake of Ca and the uptake of other essential nutrients, such as N and P.

Plant responses to ADE

In our study we found lower survival in seedlings of *Poeppigia* and *Terminalia* that were growing in ADE. Seedlings of *Poeppigia* were not able to develop a root system on ADE and the seedlings that could establish on non-ADE showed some chlorosis (E. Quintero, personal observation). These characteristics can be associated with the inability of plants to regulate Ca uptake when they grow in soils with high Ca concentration. Studies in calcifuges species have suggested that an excess of Ca in the tissues translates into Ca toxicity, that inhibits growth, and causes mortality (Jefferies and Willis 1964). This inhibition could be caused by metabolic disorders related to enzyme inactivation and to a decrease of P availability for metabolism due to the excess of cytosolic Ca (Jefferies and Willis 1964, Grundon 1972, Zohlen and Tyler 2004).

We predicted that in high-fertility ADE plants would invest in aboveground light capture, whereas in non-ADE plants would invest in belowground nutrient capture. This is in line with Brower's hypothesis which states that plants invest in capturing the resource that is in most limiting supply (Brouwer 1962). We found that seedlings growing on ADE allocated more biomass to leaves (LMF) than seedlings on non-ADE, but we did not find support for higher investment in root biomass on non-ADE. Additionally, we found higher investment on leaf area per leaf mass (SLA) and per plant mass (LAR) on ADE. Greater biomass allocation to leaves in combination with higher SLA and LAR on ADE (Table 4.2) could increase seedlings capacity to capture light and should enhance biomass growth (Poorter 1999). However, in our experiment, seedling RGR was similar on both soil types, suggesting that an increase in leaf light capture capacity does not translate into faster growth. There are other factors such as leaf tissue chemistry that could explain the limiting seedling growth found in this study (Figs. 4.4 and 4.5).

High Ca in ADE soils affect leaf nutrient stoichiometry

One of the most interesting results of our study is the effect of ADE on leaf nutrient concentrations of some of the most important elements for plant growth. ADE was more fertile, with 3.2 times more P, 2.4 times more Ca, and 1.6 times more N than the non-ADE (Appendix, Table A4.2). All species had indeed higher leaf Ca in ADE than in non-ADE (Fig. 3a), but only 6 out of 13 species had higher leaf P and leaf N on ADE (Figs 4.3b, 4.3c, 4.4a, 4.4b). Seedlings of seven species had, on average, 1.3 times higher leaf P concentrations in non-ADE than in ADE, even though ADE had more P than non-ADE soils (Appendix, Table A4.1). This result suggests that there is an imbalance between soil and tissue elements in some of the species that we studied. There are several possible explanations for this result: first, the effects of high Ca in soils and its consequences for P availability, second, the effects of a high Ca supply on plant tissue and the interaction with inorganic P, and finally, a decrease in N-fixation leading to a decrease in P uptake by N-fixer species on ADE.

Firstly, soils that combine a neutral to alkaline pH with a high Ca concentration (as it is the case in this Bolivian forest) may have, in fact, a low effective availability of P. The relatively high Ca in ADE could lead to the formation of mineral insoluble calcium phosphate, thus reducing P availability as it is reported in calcareus soils with pH around 7.8 (Lee 1998, Zohlen and Tyler 2004). In such conditions, only species that can exude organic acids into the rhizosphere to increase the solubility of phosphate would be able to incorporate more P from the soil, as it has been shown in other studies (White and Broadley 2003). Further studies on rhizosphere biochemistry and detailed information about soil P could confirm this mechanism.

Secondly, high concentrations of Ca in the cytosol of plant cells can react with inorganic P and produce calcium phosphate, decreasing the concentration of P available for plant metabolic functions and consequently, the P concentration measured in leaves (Tyler 1996, Zohlen and Tyler 2004). Unfortunately, this mechanism has been mainly studied in species that are not able to grow in calcareous soils with high soil Ca concentrations (i.e. calcifuge species). Further exploration of the interaction between Ca and P deserve exploration in the ADE context where the major changes in the chemistry of the soils is defined by increases of Ca and P (Glaser and Birk 2012).

Third, some of the species belonging to Fabaceae (e.g., *Erythrina*, *Samanea*, and *Albizia*) might have a better N-fixation and higher P uptake on non-ADE than on ADE soils. These species had either more proportion of seedlings with nodules, or a higher number of nodules per root on seedlings growing on non-ADE than in ADE (data not shown). Thus, more nodules can increase N-fixation that can increase P uptake on non-ADE (Figs 3b and 3c). Recent studies suggests that N-fixers can increase P uptake by being able to invest more carbon for either associate with mycorrhizal fungi or release phosphatase into the soil which can give them advantages to incorporate P (Nasto et al. 2014).

Leaf Ca predicts species growth, but only for those species that can cope with Ca excess

Growth of seedlings in this experiment did not appear to be nutrient limited. In both soil types, seedlings had higher leaf Ca and N concentration (Table 4.2) than reference levels normally found to sustain plant growth (5 mg g^{-1} dry mass for Ca; 15 mg g^{-1} dry mass for N) (Epstein and Bloom 2005). Leaf P concentrations were, however, close to reference values (2 mg g^{-1} dry mass) (Epstein and Bloom 2005), with some exceptions, such as *Erytrhina* (4.3 mg g^{-1} dry mass) and *Albizia* (4.5 mg g^{-1} dry mass) (Fig. 4.3b). But this “luxurious” nutrient consumption of Ca, N and P did not translate into higher RGR of the species (Fig. 2b). Lack of changes in plant growth with increased nutrient availability in ADE seems to be associated with species-specific abilities in tissue nutrient regulation. Whereas some species increased RGR when leaf Ca, P and N increased simultaneously (Figs. 4.5a-d), other species increased leaf Ca with no consequences on leaf N or P and RGR (Figs. 4.5a-d). This implies that increases of Ca in the soils and in plant tissues are not beneficial for some species. In general, Ca enters in the plants by diffusing among cells or through cell cytoplasm in the roots, then it goes to the xylem and it accumulates in the leaves (White 2012). Plants that are not adapted to high Ca in the soils (such as calcifuge plants) cannot regulate the excess of Ca entering in their tissues. This excess of Ca uptake is done at the expense of the uptake of other ions, such as such as K, Fe and Mg, causing nutritional imbalance (Jefferies and Willis 1964). We cannot confirm if our plants had a nutritional imbalance, since we did not measure other nutrients in the leaves, although we did not notice any symptoms of nutrient deficiency. Additionally, excess of Ca can induce some metabolic problems. The excess of cytosolic Ca can decrease cellular free P necessary for metabolic functions (Zohlen and Tyler 2004) or it can inhibit some enzymatic action by competing with enzyme cofactors, such as Mg and Mn (Jefferies and Willis 1964, Webb 1999). Unless plants are able to regulate the excess of Ca through storing it in the vacuoles, in the cell wall (Kinzel 1989), in epidermal cells or in structures such as trichomes (Webb 1999), it is very likely that Ca can produce some metabolic dysfunctions. This has been suggested in studies that report poor plant development on soils with high concentrations of Ca (Jefferies and Willis 1964, Lee 1998).

Trees at La Chonta do not seem nutrient limited since natural soils are fertile in terms of N, P, Ca and pH values. The improvement to the soil by increasing Ca and P levels, as it is the case in ADE, does not translate in higher tree RGR, and it can be even detrimental for some tree species such as *Terminalia* and *Poeppigia* that seemed to suffer from Ca toxicity. Our results suggest "luxury" consumption of Ca by tree seedlings, but the advantages of this consumption depend on the plant ability to maintain the balance among the various nutrients needed for performance. On one hand, it is likely that the species that did not increase RGR on ADE, such as *Samanea, Erythrina* and *Albizia* are unable to regulate excess of Ca and show some nutrient imbalance. On the other hand, species that had a higher RGR in ADE soils , such as *Cedrela*, and *Sapindus*, are able to regulate the excess of Ca and increase their leaf P and N concentrations on ADE as well. Nevertheless, we did not elucidate the exact mechanism of Ca accumulation or detoxification (He et al. 2014) .

Concluding remarks

Past inhabitants of the La Chonta forest modified the soils by increasing mostly Ca and P concentrations in ADE. Contrary to our expectation, this soil enrichment did not lead to a general increases in tree growth, probably because the background soil fertility of non-ADE at La Chonta is already high. Our results indicate three possible ways of regulating increase of

Ca in the soil. Therefore, the ability of plants to colonize and grow in patches of ADE depends on their ability to cope with increases in soil nutrients, especially increases in Ca.

In the Amazon basin, sites where the natural soils are nutrient limited, ADE soils can improve conditions for plant growth; whereas at sites where nutrients in the soils are not limited, ADE potentially could cause nutrient imbalance and general nutritional disorders that do not improve plant growth.

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Appendix.

Laboratory procedures for obtaining soil chemical and physical variables

The concentrations of N, P, exchangeable Ca^{2+}, Mg^{2+}, K^+, and Al^{+3}; pH; organic matter content (OM); total exchangeable bases (TEB); cation exchangeable capacity (CEC); base saturation; acidity; and soil texture were analysed. Soil texture (percentage of sand, silt, and clay) was measured using a Bouyoucos hydrometer; pH and electric conductivity was determined in 1:5 distilled water equilibrating for 90 min.; concentrations of exchangeable Ca^{2+}, Mg^{2+}, K^+ and Na^+ were determined in 1 M ammonium acetate at pH 7. After extraction, Ca^{2+} and Mg^{2+} were measured with an Atomic Absorption Spectrophotometer; concentrations of K^+ and Na^+ were measured with a flame photometer; exchangeable aluminium (Al^{3+}) was measured in 1 M hydrochloric acid (HCl). Total P was determined by Olsen method; OM was determined by Walkley-Black method, and total N was measured using the Micro-Kjeldahl method (Barber and Cochrane 1993). TEB was defined as the sum of the most important exchangeable cations (Ca^{2+}, Mg^{2+}, K^+ and Na^+). CEC was calculated as the sum of TEB plus acidity. Base saturation was computed as the percentage of exchangeable cations or bases relative to total CEC.

Table A4.1 Soil characteristics of Amazonian Dark Earths and non-Amazonian Dark Earths collected in La Chonta forest concession, before and after mixing them with river sand. Ratios between the values of the variables after mixing with river sand are presented.

	ADE	**non-ADE**	**ADE / River sand**	**non-ADE / River sand**	**Ratio variables on ADE:nonADE**
pH	7.4	7	7.8	7.6	1
Electric conductivity (µS/cm)	226	108	218	149	1.5
Ca (cmol kg^{-1})	12.2	4.8	10	4.2	2.4
Mg (cmol kg^{-1})	1.3	1	1.1	0.8	1.4
Na (cmol kg^{-1})	0.11	0.04	0.09	0.05	1.8
K (cmol kg^{-1})	0.38	0.26	0.28	0.2	1.4
P (mg kg^{-1})	36	12	35	11	3.2
Total N (%)	0.31	0.16	0.18	0.11	1.6
Total Exchangable bases	14	6.1	11.5	5.3	2.2
Cation Exchange Capacity	14	6.1	11.5	5.3	2.2
Base Saturation (%)	100	100	100	100	1
Organic Mater (%)	3.3	2.7	2.9	2.2	1.3
Sand (%)	49	57	68	73	0.9
Silt (%)	39	36	25	20	1.2
Clay (%)	12	7	7	7	1

Table A4.2 Trait averages for all species on each soil type. RGR= Relative growth rate; RMF=Root mass fraction; SMF=Stem mass fraction; LMF=Leaf mass fraction; LDMC=Leaf dry mass content; SLA=Specific leaf Area; LAR=Leaf area ratio; SRL=Specific root length; RLR=Root length ratio; RDMC=Root dry mass content; SDMC= Stem dry mass content.

	RGRbiomass (g.g d^{-1})		**RMF (g g^{-1})**		**SMF (g g^{-1})**		**LMF (g g^{-1})**		**Diameter of secondary roots (mm)**	
Species	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE
Hymenaea courbaril	0.002	0.004	0.20	0.21	0.30	0.31	0.50	0.48	0.71	0.67
Machaerium villosum	0.006	0.007	0.27	0.30	0.28	0.29	0.47	0.46	0.42	0.44
Ormosia nobilis	0.009	0.009	0.23	0.23	0.27	0.27	0.49	0.48	0.49	0.59
Samanea tubulosa	0.014	0.012	0.32	0.32	0.29	0.30	0.41	0.41	0.42	0.42
Erythrina crista-galli	0.016	0.014	0.19	0.22	0.33	0.37	0.48	0.41	0.70	0.66
Albizia niopoides	0.016	0.015	0.31	0.30	0.16	0.18	0.53	0.52	0.29	0.27
Enterolobium contortisiliquum	0.017	0.017	0.30	0.31	0.21	0.25	0.49	0.44	0.54	0.55
Anadenanthera colubrina	0.018	0.022	0.25	0.27	0.19	0.16	0.57	0.57	0.39	0.36
Cariniana ianerensis	0.017	0.019	0.24	0.22	0.28	0.30	0.49	0.48	0.67	0.66
Sapindus saponaria	0.021	0.018	0.20	0.21	0.25	0.24	0.51	0.49	0.41	0.53
Cedrela fissilis	0.023	0.019	0.30	0.34	0.26	0.23	0.46	0.42	0.52	0.52
Swietenia macrophylla	0.025	0.024	0.12	0.11	0.27	0.29	0.63	0.65	0.38	0.37
Chorisia speciosa	0.027	0.027	0.10	0.10	0.24	0.24	0.62	0.60	0.45	0.45

Trait (continuation)	LDMC (g g^{-1})		Stem Density (g cm^{-3})		SLA (cm^2 g^{-1})		LAR (cm^2 g^{-1})		Force to punch (N mm^{-1})	
Species	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE
Hymenaea courbaril	0.32	0.34	0.33	0.45	202.32	203.11	97.68	93.35	0.26	0.28
Machaerium villosum	0.31	0.32	0.30	0.30	252.36	240.80	110.28	102.47	0.30	0.33
Ormosia nobilis	0.28	0.29	0.19	0.20	305.35	307.02	134.36	134.05	0.31	0.30
Samanea tubulosa	0.28	0.30	0.40	0.37	525.76	487.05	187.16	171.84	0.19	0.22
Erythrina crista-galli	0.14	0.14	0.15	0.13	541.99	516.19	206.80	171.45	0.17	0.18
Albizia niopoides	0.34	0.36	0.53	0.53	388.68	388.71	191.75	188.98		
Enterolobium contortisiliquum	0.25	0.26	0.41	0.38	500.48	451.96	188.45	151.80		
Anadenanthera colubrina	0.49	0.49	0.57	0.50	282.29	250.48	148.71	133.40		
Cariniana ianerensis	0.23	0.25	0.22	0.26	355.07	305.40	167.82	138.88	0.35	0.32
Sapindus saponaria	0.33	0.33	0.48	0.52	344.63	329.73	177.21	162.52	0.23	0.26
Cedrela fissilis	0.19	0.21	0.16	0.17	648.52	545.40	249.91	199.79	0.13	0.16
Swietenia macrophylla	0.24	0.24	0.19	0.21	392.67	410.15	231.04	248.64	0.25	0.26
Chorisia speciosa	0.11	0.11	0.09	0.09	610.36	609.98	322.78	315.95	0.14	0.17

Trait (continuation)	Number of Secondary roots		SRL (cm g^{-1})		RLR (cm g^{-1})		RDMC (g g^{-1})		SDMC (g g^{-1})	
Species	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE
Hymenaea courbaril	14.33	15.90	1043.63	779.28	42.85	36.80	0.23	0.27	0.33	0.36
Machaerium villosum	13.92	18.55	2605.38	1999.71	792.89	347.29	0.24	0.25	0.34	0.35
Ormosia nobilis	26.50	21.17	850.40	1139.80	138.52	150.42	0.21	0.22	0.27	0.27
Samanea tubulosa	42.50	41.17	432.81	568.52	228.57	319.78	0.18	0.21	0.36	0.33
Erythrina crista-galli	28.18	36.00	1581.65	1297.06	304.50	279.89	0.10	0.11	0.15	0.16
Albizia niopoides	29.25	29.33	1975.07	1536.77	8106.05	5041.89	0.17	0.19	0.37	0.37
Enterolobium contortisiliquum	36.36	38.73	3570.32	2111.67	48.46	19.67	0.17	0.18	0.32	0.32
Anadenanthera colubrina	12.83	26.33	2099.25	1590.84	499.74	423.14	0.23	0.26	0.38	0.41
Cariniana ianerensis	27.13	28.17	3998.30	3519.98	86.55	82.74	0.15	0.16	0.25	0.30
Sapindus saponaria	51.11	42.40	695.26	1056.07	28.76	65.32	0.21	0.22	0.41	0.43
Cedrela fissilis	30.42	29.75	2582.54	2601.03	745.21	880.43	0.12	0.13	0.16	0.18
Swietenia macrophylla	46.25	47.08	526.64	1250.33	61.14	134.73	0.16	0.16	0.23	0.23
Chorisia speciosa	23.50	20.33	1878.84	1502.24	1205.45	1345.07	0.09	0.09	0.11	0.11

Trait (continuation)	Leaf thickness (mm)		Leaf Ca content (mg g^{-1})		Leaf N content (mg g^{-1})		Leaf P content (mg g^{-1})		Ca:P	
Species	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE
Hymenaea courbaril	0.14	0.13	0.80	0.65	2.14	2.17	0.21	0.23	3.83	2.88
Machaerium villosum	0.16	0.17	2.09	1.81	2.97	3.04	0.12	0.11	17.05	17.28
Ormosia nobilis	0.15	0.14	1.17	0.90	2.63	2.62	0.14	0.13	8.39	7.09
Samanea tubulosa	0.09	0.09	1.56	1.52	4.70	4.96	0.11	0.15	14.42	11.59
Erythrina crista-galli	0.14	0.14	2.33	1.71	3.97	4.41	0.19	0.43	12.05	3.98
Albizia niopoides			2.44	1.71	4.65	4.93	0.41	0.45	6.27	3.78
Enterolobium contortisiliquum			0.66	0.55	4.62	4.74	0.16	0.21	4.27	2.70
Anadenanthera colubrina			2.04	2.01	3.53	3.34	0.16	0.14	12.79	14.63
Cariniana ianerensis	0.10	0.11	1.61	1.39	1.82	1.83	0.12	0.13	13.36	11.54
Sapindus saponaria	0.08	0.08	2.63	2.31	2.87	2.77	0.22	0.22	11.81	11.07
Cedrela fissilis	0.10	0.10	2.30	2.21	4.11	3.84	0.18	0.15	12.45	16.20
Swietenia macrophylla	0.11	0.11	2.27	2.18	2.54	2.48	0.16	0.17	14.26	12.94
Chorisia speciosa	0.15	0.15	3.08	2.95	4.52	4.89	0.25	0.23	12.80	13.62

Trait (continuation)	**Ca:N**		**N:P**		**Leaf Production (leaves d^{-1})**	
Species	ADE	non-ADE	ADE	non-ADE	ADE	non-ADE
Hymenaea courbaril	0.37	0.31	10.24	9.49	0.02	0.04
Machaerium villosum	0.70	0.60	24.39	28.67	0.02	0.02
Ormosia nobilis	0.45	0.34	18.90	20.69	0.02	0.02
Samanea tubulosa	0.33	0.30	42.90	33.47	0.07	0.06
Erythrina crista-galli	0.59	0.39	20.53	10.22	0.09	0.09
Albizia niopoides	0.53	0.35	11.34	10.94	0.05	0.05
Enterolobium contortisiliquum	0.14	0.12	29.12	22.44	0.07	0.06
Anadenanthera colubrina	0.58	0.60	21.47	24.25	0.05	0.05
Cariniana ianerensis	0.88	0.76	15.06	14.11	0.09	0.08
Sapindus saponaria	0.91	0.84	12.87	12.87	0.08	0.07
Cedrela fissilis	0.56	0.58	22.24	25.66	0.05	0.03
Swietenia macrophylla	0.90	0.88	15.93	14.70	0.08	0.09
Chorisia speciosa	0.70	0.61	18.22	21.06	0.12	0.09

Chapter 5

General Discussion

Estela Quintero-Vallejo

Introduction

The myth of tropical forests as pristine and untouched ecosystems is rapidly changing in favour of the idea that large extensions of tropical forest are rather old growth forests that could have likely gone through human influence in the past (Willis et al. 2004, Ghazoul and Sheil 2010). Processes of permanent inhabitation, expansion and retreat of human populations have not always been obvious in those ecosystems, leaving sometimes weak and overlooked imprints in the landscape (Bush and Silman 2007, Carson et al. 2014). However, a growing list of evidence reveals important modifications generated by humans in the Amazonian region, ranging in scale from scant openings in the forest to changes in the entire landscape (McMichael et al. 2012, Heckenberger 2013). Whereas regions in central Amazon basin and sites close to rivers were more intensively and extensively inhabited (Erickson 2008), there is evidence that in western Amazonian region humans created only sparse clearings in their settlements for agriculture (McMichael et al. 2012, Urrego et al. 2013). Colossal earthworks and massive movement of earth to make artificial mounds, were used to protect small villages from enemies and for cultivation in inundated sites, for example in the region of Llanos de Moxos, Bolivia (Erickson 2006, Erickson 2010, Lombardo and Prümers 2010, Dickau et al. 2012, Whitney et al. 2013). In savannahs, humans used fire to eliminate trees and allow agricultural activities (Carson et al. 2014). Across several sites in the Amazon basin, the opening of the forest for cultivation (Arroyo-Kalin 2012) or the introduction and enrichment of useful species such as palms and trees, were common practices (Clement 1999a). These modifications have produced legacies that, in one way or another, are currently reflected in the forest (Levis et al. 2012, Chazdon 2014).

Besides of the more or less noticeable aboveground effects of human occupation in tropical forests, less visible belowground changes in the soil are also part of the footprint of humans in these environments. One of these soil modifications, referred here as Amazonian Dark Earths (ADE), is the product of the accumulation of pieces of charcoal, organic matter and some elements, such as P, Ca, and N, that changed soil conditions and favoured agricultural practices (Glaser and Birk 2012, WinklerPrins 2014). Studies that measured crop productivity in fallows have already established that ADE increase soil fertility (German 2003), but little is known about the effect of increased soil fertility in natural forests (Junqueira et al. 2010, Doughty et al. 2014). The aim of this thesis is to evaluate the effects of soil changes due to ADE on the forest that has grown after abandonment by indigenous populations declined or abandoned the areas. First, I characterized the soils to assess the magnitude of the changes in the soil as a consequence of human occupation. Then, I studied how soil changes affected species composition in the understory (Chapter 2) and forest dynamics (Chapter 3), and how plants respond to anthropogenic changes in soil properties (Chapter 4). In the current chapter, I synthesize the results of this thesis and discuss how the acquired knowledge helps to contextualize the phenomenon of ADE in the Amazonian forest and its contribution to the discussion of the creation of ADE. Besides, I discuss the extent to which ADE corresponds to a human legacy in the forest. Finally, I discuss the results of this thesis regarding to the effects of increased soil pH in the understory species composition, forest structure and dynamics, and plant responses to changes in soil Ca and pH.

Soils in the Amazonian region, fertility in the south-west and ADE in context

Recent soil characterizations of the whole Amazonian basin have reported high variation in soil composition and properties (Quesada et al. 2011). This variation is mainly caused by the diverse geological history of the region (Hoorn et al. 2010), which determines soil fertility. Thus, soils from the older regions in the eastern and central Amazonian forest are less fertile, with a lower concentration of available and total P and lower total base content (sum of Ca, Mg, Na, K) than soils from younger regions in the south-west.

Our study site in the lowlands of Bolivia, is located in a region where soils have been classified as Ferralsols and Lixisols, which are usually identified as ancient poor soils (Quesada et al. 2011, Gardi et al. 2014). The classification in Quesada et al (2009, 2011) has been based on the world soil database (SOTERLAC-ISRIC -version 2.0, 1.5 million scale). In this thesis, a detailed description of soil profiles allowed us to propose a reclassification of the soils at La Chonta as a combination of Cambisols, Histosols/Gleysols, Leptosols and Anthrosols, which in general are soils that possess high nutrient concentrations (See appendix; Gardi et al. (2014)). Discrepancies between our characterization and current soil classification can be the result of the non-formal, published descriptions of the soils of this area, based on only one site about 300 km north-east from La Chonta (Noel Kempff Mercado National Park) (Quesada et al. 2011). I did not find Ferralsols in La Chonta, despite their alleged presence in the area. Ferralsols are mainly associated with old-aged soils, but in La Chonta, soil history seems to be more complex because of the geological and geomorphological processes of the region. Thus in La Chonta, there is a mixture of old and young soils originated from the south corner of the ancient Precambrian Brazilian shield; the more recent fluvial sediments caused by the uplifting of the Andes (Hoorn et al. 2010, Latrubesse et al. 2010); flooding and drought events during the mid-Holocene (Taylor et al. 2010, Urrego et al. 2013); and modifications of the soil due to human occupation in the area around 400 yr BP (Paz-Rivera and Putz 2009). Thus, further soil surveys in the region will produce a more accurate classification of the soils in La Chonta and the surroundings area, that complement the information presented in this thesis.

A previous study found that *ca.* 20% of a surveyed area of 216 ha in La Chonta contained Anthrosols, '*terra preta*' or ADE (Paz-Rivera and Putz 2009), indicating that these soils are relatively common in the forest. Although the World Reference Base of Soil Classification has recognized '*terra preta*' as a type of Anthrosol, there is still debate about their unique characteristics, mainly because of the high variability of the soil parameters between and within archaeological ADE sites (Arroyo-Kalin 2012, Glaser and Birk 2012, Schmidt et al. 2014). Although this discussion is beyond the scope of this thesis, the information presented here might contribute to future considerations regarding the classification of ADE.

Most studies in the Amazonian region have found large differences in soil color, pH, organic matter content, CEC and concentrations of nutrients, such as P, Ca, N, Mg, K and Zn, between ADE soils and surrounding soils (Glaser and Birk 2012). Particularly, P and Ca concentrations are hundreds and even thousands of times higher in ADE soils than in adjacent natural soils (Glaser and Birk 2012). However, variability in the concentration of P between and within ADE sites can also be high (Lehmann et al. 2003b, Falcão et al. 2009)(Fig. 5.1). Interestingly, differences in P, N, Ca, K and Mg concentrations between ADE and neighbouring soils in La Chonta, are lower (about 10 times) than the differences found in other sites in the Amazonian region where the largest differences can be more than 100 times, as illustrated in Fig. 5.1 (that includes information from Chapter 2). The same pattern holds true for soil pH; differences in soil pH between ADE and non-ADE sites were less pronounced in La Chonta (increase by 0.4 units) than in other sites in the Amazonian region (increase of around 1 unit, Table 5.1). Our data suggest that, at a larger scale, soil modification through ADE in La Chonta seems not to be as intense as in other sites in central Amazonian expanse because the underlying soil fertility in La Chonta is already high. However, La Chonta is not unique in this respect, as other studies north of our site in Baures and Huacaraje municipalities (province of Iténez, Bolivia) have also reported modest contrasts between ADE and adjacent soils (Hastick et al 2013). Additional evidence of the similarity between Anthrosols and natural soils in La Chonta comes from Principal Component Analyses (PCA) on soil characteristics described in Chapter 3. In the PCA, soil samples where we found ceramics are interspersed with soil samples without ceramics (Fig. 5.2), indicating that human activities have not always enhanced soil fertility or that ceramic remains are not always found where human activities have changed soil fertility. Since our survey of the occurrence of ceramics was not designed to rigorously assess the extension of settlement sites, we cannot be strictly certain about the extension of ADE in La Chonta. Nevertheless, our PCA can be interpreted in three alternative ways: First, the area of soil modification was large (we sampled over 324 ha dispersed in an area of around 33.7 km^2). This is consistent with findings of wide spread ADE in La Chonta (around 20%) (Paz-Rivera and Putz 2009), and evidence of long and extended history of human occupation recorded in Llanos de Moxos (north from our site in Bolivia) (Erickson 2006, Lombardo and Prümers 2010, Walker 2011, Lombardo et al. 2013). Second, natural soils in La Chonta have a higher and variable amount of nutrients and the addition of minerals by human occupation has not substantially changed soil fertility. Finally, it is also possible that human habitation of the area was not long enough to make more substantial transformations to the soil. Further archaeological studies are needed to clarify the history of habitation patterns in this forest.

There are some discrepancies between this PCA (Fig. 5.2) and the one showed in Chapter 2 (Fig. 2.1). Whereas in Fig. 2.1 each data point consisted of a compound sample of a soil core taken every 25 m in a 150 m transect, in Fig. 5.2 each point corresponds to a unique soil core taken somewhere in the 12 permanent plots. The latter sampling scheme possibly captures a wider range of soil variability within ADE sites, a factor that needs to be considered when characterizing ADE soil properties (Schmidt et al. 2014). Besides, the high fertility of the underlying soils of La Chonta, can make difficult a clear separation among the points of Fig. 5.2.

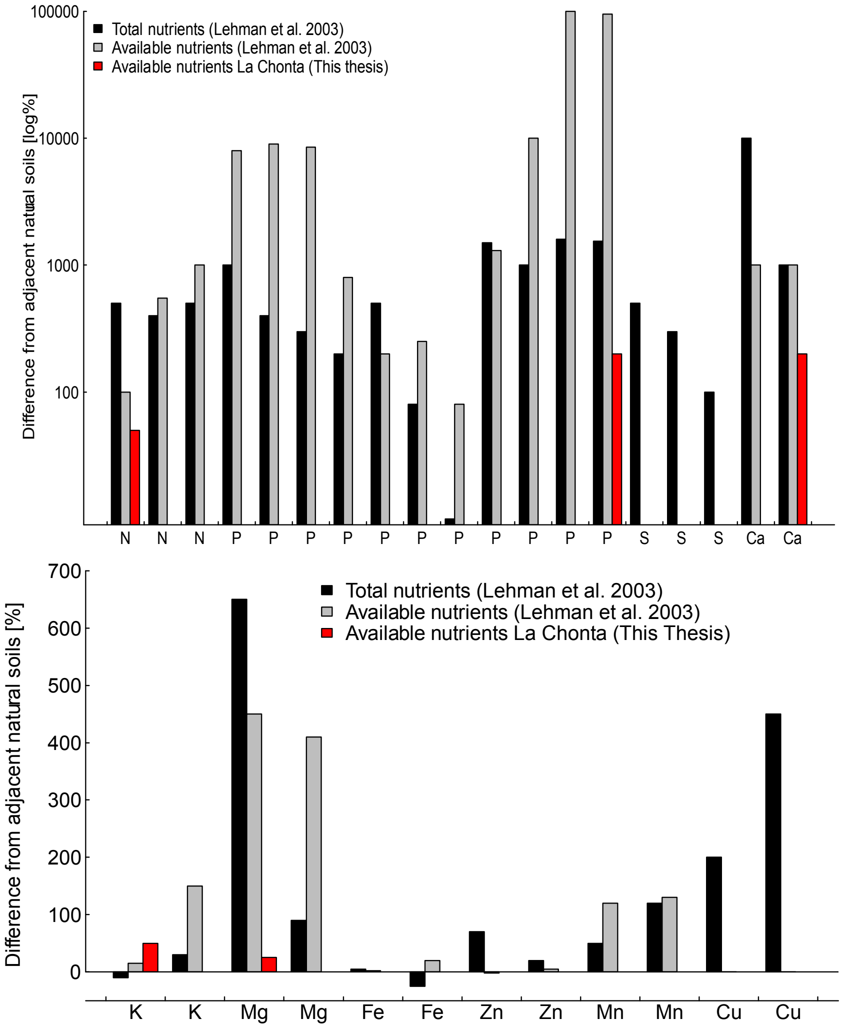

Figure 5.1 Differences in percentage of nutrients between ADE and adjacent natural (mostly Ferralsols) soils in several Amazonian sites, mostly in Central and Eastern Amazonian region (where most natural soils are Ferralsols). Modified from Lehmann et al. (2003). $Difference[\%]_{ADE-nonADE} = \frac{Nutrient\ content_{ADE} - Nutrient\ content\ non_{non-ADE}}{Nutrient\ content\ non_{non-ADE}} x100[\%]$. I included my own data from La Chonta as red bars. The calculated difference of our soils in La Chonta correspond with the average of 36 transects (18 in ADE with presence of ceramics and 18 non-ADE) in La Chonta forestry concession. Differences for P=172,7; Ca=159,4; N=45,3; Mg=12,6; K=62,0 % (Chapter 2).

Table 5.1 Values of soil pH (based on H_2O) from different sites distributed through the Amazon River Basin, including La Chonta. The sites are ordered from lowest to highest pH values.

Study site	**pH (H_2O)**		**Reference**
	ADE	**Non-ADE**	
Araracuara, Amazon Department (Colombia)	4.8	4.4	(Eden et al. 1984)
Costa de Açutuba (Brazil)	5.7	4.8	(Falcão et al. 2009)
Fazenda Juquitaia (Brazil)	5.5	4.8	(Falcão et al. 2009)
Costa do Laranjal (Brazil)	6.0	5.0	(Falcão et al. 2009)
Hatahara, close to Manaus (Brazil)	6.6	4.7	(Falcão et al. 2009)
Iranduba District (Brazil)	5.9	4.6	(Lima et al. 2002)
Tranquilidad and Embolla, Iténez Province (Bolivia)	6.8	5.8	(Hastik et al. 2013)
La Chonta, Guarayos Province (Bolivia)	7.1	6.6	(Quintero-Vallejo et al. 2015)
La Chonta, Guarayos Province (Bolivia)	7.4	7.0	This thesis Chapter 4

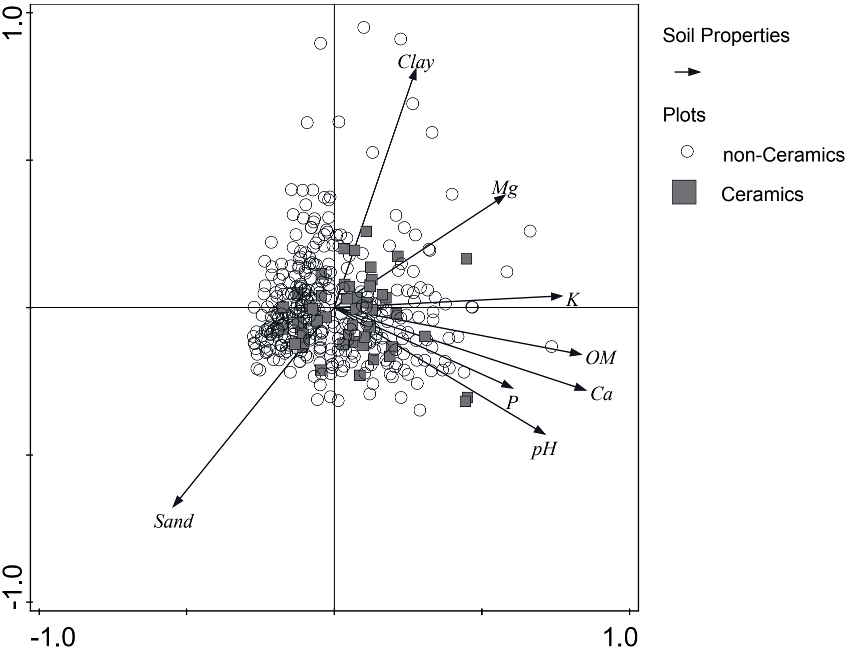

Figure 5.2 Principal Component Analysis of eight soil properties of 398 soil samples collected from12 permanent plots in La Chonta forestry concession, Guarayos Province, Bolivia. For details of soil sampling and analyses see Chapter 3 (Appendix 3.1). The presence of ceramics in the soil sample is indicated (filled squares = ceramics found, open circles=no ceramics found). Presence of ceramics was not included in the variables to run the analyses, it was only used to distinguish the points. P correspond to values of available phosphorus.

In summary, I found that differences in soil composition between natural soils and ADE at La Chonta were not large when we compare them with other sites of the Amazon basin. Still, it seems that past human settlements in La Chonta resulted in the addition of minerals, such as P and Ca that enhanced soil nutrients and soil pH (Appendix, Chapter 2). In the next sections, I will examine the effect of these changes on important forest attributes, such as forest composition and community dynamics.

Effects of soil nutrient concentration on forest composition, forest dynamics, and plant growth.

Species composition

Soil nutrients play an important role in shaping forest composition and dynamics. The concentration of soil nutrients explain differences in species composition of understory herbs (Costa 2006), ferns (Tuomisto et al. 2014, Zuquim et al. 2014) and tropical trees (John et al. 2007, Toledo et al. 2011b, Baldeck et al. 2013, Condit et al. 2013). However, studies that relate species composition with soil characteristics rarely include changes in soil properties as a consequence of past human modification and, if they do, they focus on species that are useful for humans (Clement et al. 2009, Paz-Rivera and Putz 2009, Levis et al. 2012, Ross et al. 2014). The only study that did not target useful species only, still found that species composition of ADE sites was characterized by high abundance of useful and domesticated species (Junqueira et al. 2010).

In this thesis, I studied how plant species respond to soil nutrient concentrations, by focusing both on species that are little or not used by humans, such as herbs and ferns and species that are used by humans (for example palms) to try to separate effects associated with the intentional cultivation of species from effects associated with plant responses to soil changes, as is the case of ferns (Tuomisto et al. 2014) (see Chapter 2). I found that changes in soil pH (which most likely indicates an increase in soil Ca) affected the composition of fern, herb and palm species that inhabit the understory, and that it decreased fern diversity (Chapter 2: Table 2.2, 2.3, Fig. 2.3). Although I did not explore in full detail the underlying mechanisms, it seems that fern species respond to changes in the concentration of soil nutrients Chapter 2: Fig. 2.3) (Amantangelo and Vitousek, 2008). Changes in the abundance of palms might be determined by the history of human populations in the region. For example, the occurrence of the palm *Astrocaryum aculeatum* has been associated with past human settlements in La Chonta and other sites in central Amazonian region (Major et al. 2005a, Paz-Rivera and Putz 2009). Palms and other domesticated species are recognized as species that have been intensively used by inhabitants of the Amazon basin in the past (Clement 1999a, b, Morcote-Ríos and Bernal 2001), and recent research provides evidence of the influence of humans in their current distribution (Levis et al. 2012). However, we lack information on the effects of human-modified soils on the current distribution of plants in the Amazonian forest. Research done in other regions of the world indicate human-induced soil modification effects on plant distribution (Dambrine et al. 2007). For example, shell middens left by ancient inhabitants in the East coast of the United States of America led to a shift from less woody to a more herbaceous community as a result of a particular composition of soil nutrients (Cook-Patton et al. 2014). This thesis shows that even small changes in soil properties of a place located in the south-western Amazonian region changed understory species composition (Chapter 2). It would be interesting to explore whether large changes in soil properties, as those observed in ADE in the central Amazonian expanse, would have similar effects.

In summary, the legacies of human occupation on the composition of plant species in the La Chonta forest seem to be the result of an interplay between changes in soil made by humans and the modification in the distribution of those species used by humans (Fig. 5.3).

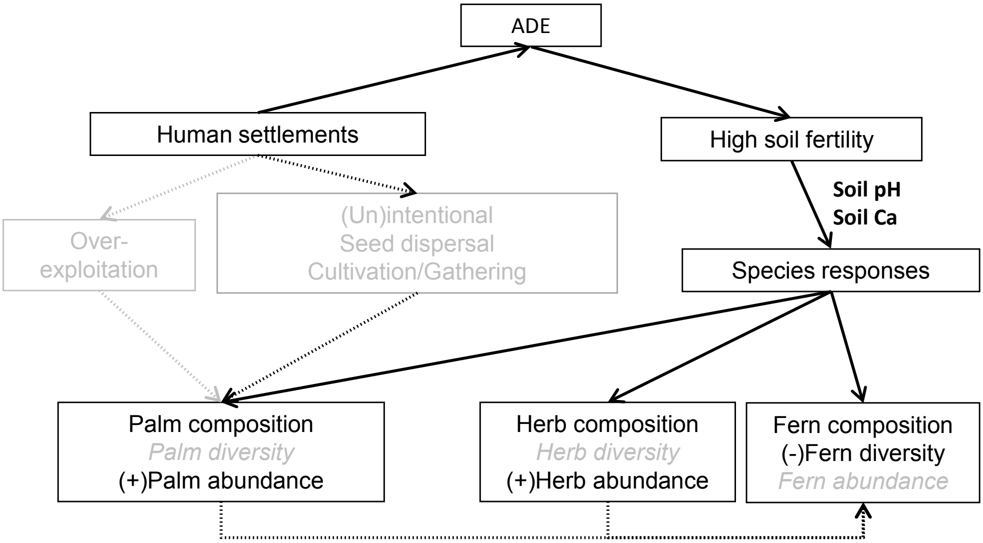

Figure 5.3 Conceptual diagram of human legacies on understory species composition. ADE is associated with changes in species composition through increases in soil Ca and soil pH that affect species composition of ferns, herbs and palms, and negatively affect fern diversity. Grey boxes correspond to factors that we did not measure but we recognize that it could have an effect on the attribute. Grey text in *italics* correspond to attributes for which we did not find a significant effect. Continuous arrows correspond to the attributes for which we found effects and dashed arrows correspond to attributes for which we found a possible effect but we did not evaluate this directly. Signs in parentheses show the direction of the effect; (+) indicates a positive effect and (-) indicates a negative effect.

Forest structure, dynamics and plant growth

In the Neotropics few studies have found significant relationships between nutrient concentrations and forest structure at a local scale (Laurance et al. 1999, DeWalt and Chave 2004). At small scales, forest structure can be associated with soil characteristics such as available P in the soil (Paoli et al. 2008) but also with endogenous processes such as gap formation (Ruslandi et al. 2012) and with human perturbation (Peña-Claros et al. 2008, Bourland et al. 2015). Few studies have looked at forest structure on ADE. One of those studies looked at younger forests (~30 yr old) growing on ADE (Junqueira et al. 2010). They found that forest on ADE had lower tree density at early stages of the forest succession than similar aged forest on non-ADE. This could be caused by high dominance of fast grow species that rapidly colonize and establish on high nutrient sites (Junqueira et al. 2010) as it has been suggested by high dominance of annual herbaceous species in ADE sites close to Manaus city (Major et al. 2005a). Besides, older forests on ADE are more productive than forest on non-ADE, i.e. they have higher net primary productivity (Aragão et al. 2009, Doughty et al. 2014). So, it seems that over both relatively short and long terms ADE has an an effect on forest structure.

I studied the effect of ADE on forest attributes and components of forest dynamics, such as tree growth, mortality and recruitment (Chapter 3) and found weak soil effects on forest structure and dynamics. I expected that concentration of nutrients, such as P would be the main determinant of forest structure. Surprisingly, I found that soil pH and not P emerged as the soil property that consistently had a significant effect on forest structure and dynamics (Chapter 3: Figs. 3.2, 3.3; Fig. 5.4). Soil pH had a positive effect on tree recruitment, a negative effect on tree mortality of the plot, and a positive or negative effect on tree basal area of the plot, depending on the tree size class considered (Chapter 3: Fig. 3.2).

One remarkable aspect of soils in La Chonta, is that they have, in general, high pH values in comparison with other soils in the Amazon basin (Table 5.1; Table 3.1). Many tropical forest grow in sub-optimal acid soil conditions. Therefore soil amendments to increase pH, such as addition of ashes or charcoal (Glaser et al. 2002) will be beneficial for tree growth because nutrients such as P become more available in less acid soils. Additionally, Al cations, which can be toxic for plants will become less available in the soil (Nkana et al. 1998). Nevertheless, Ca addition in ADE soils in La Chonta increased soil pH to neutral or higher values (Chapter 3: Table 3.1). Although theoretically the increase in pH would enhance tree growth, I argue that high values of pH could result in supra-optimal conditions. Studies that have explored the effect of increases of soil pH beyond neutral values are scarce. One such a study, added wood ash to forest stands (Augusto et al. 2008). Addition of ashes to acidic soils increased soil pH and, in the long term, increased the availability of nutrients such as Fe, Ca, P, thus favouring tree growth. However, when ashes were added to soils with a pH close to 7, tree growth did not increase (Pitman 2006, Augusto et al. 2008). Moreover, the response of trees to an increase in soil pH seems to be species-specific. In temperate forests it has been shown that broadleaved species perform better in higher pH soils and have, in general, higher nutrient uptake of K, Ca and Mg than coniferous species (Vance 1996). Although rigorous comparisons have not yet been made in more diverse tropical forests, the observed relationships between the availability of minerals in the soil and species composition suggests that different species respond to soil resources in a different way (John et al. 2007, Baldeck et al. 2013). This was confirmed in Chapter 3 (Table 3.2, Figs. 3.3, 3.4), where the dominance of shade-tolerant species increased with soil pH and the dominance of (long-lived) pioneers decreased with soil pH.

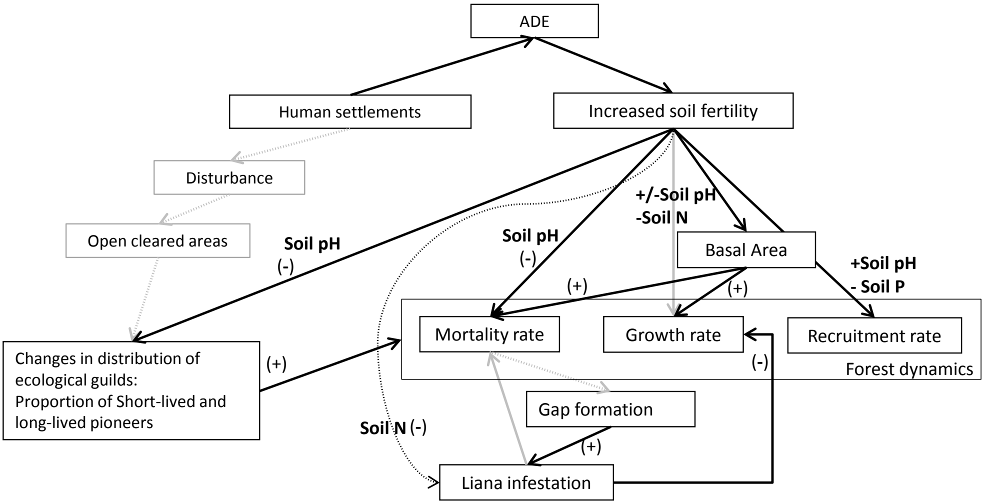

Figure 5.4 Conceptual diagram of human legacies on forest dynamics in La Chonta. Amazonian Dark Earths (ADE) affect forest dynamics by increasing soil pH and the concentrations availability of P and N. Successional guilds composition (i.e. the distribution of individuals such as pioneer and shade-tolerant species) can influence forest dynamics (i.e. a higher proportion of pioneers led to increased basal area growth rate of the plot). However, we also found that soil pH was negatively related with the successional guild composition of forest stands (Chapter 3: Fig. 3.2, Fig. 3.3) with a lower proportion of pioneers on soils with high pH. Liana infestation was positively affected by light and negatively affected by soil N. Liana infestation had, in turn, a negative effect on tree growth rate (Chapter 3; Figs 3.2, 3.3). Current forest successional composition seems a consequence of past human perturbation (Chapter 3). Black arrows indicate processes that were affected by soils or human settlements, grey solid arrows indicate processes for which no direct effect of soils was observed, and dashed grey arrows indicate processes that were not evaluated but could have an effect. Grey boxes correspond to factors that we did not measure but we recognize that it could have an effect on the attribute. Signs in parenthesis show the direction of the effect; (+) indicates a positive effect, and (-) indicates a negative effect.

Moreover, I have shown in Chapter 4 that seedlings of 17 species responded differently to ADE soils. Surprisingly, ADE had a negative rather than a positive effect on seedling growth of *Terminalia oblonga* and *Poeppigia procera*, and changed the nutrient balance in 9 other species (Table 5.2; Chapter 4: Fig. 4.4). At first sight it seems paradoxical that nutrient addition to soil has a negative effect on plant performance and functioning, but these negative effects could be explained by the possible change of either soil stoichiometry and soil pH, or the internal plant nutrient stoichiometry, emanating from the Ca addition to the soils (Chapter 4). In a greenhouse experiment I found that in spite of higher P, N and Ca concentration in ADE (Chapter 4: Appendix, Table A4.1), only 6 out of 13 species had higher leaf P and leaf N on ADE but all species had higher leaf Ca on ADE (Chapter 4: Figs 4.3b, 4.3c, 4.4a, 4.4b). These results suggested that there is an imbalance between soil and tissue elements in some of the species that we studied when they grow on ADE. These imbalances could be caused by reduced P availability for plants at high pH because reactions between soil P and Ca result in insoluble salts, as has been found in calcareous soils (Lee 1998, Zohlen and Tyler 2004). Alternatively, the nutrient inbalance in the plant is causedr by reactions between inorganic P and Ca in the interior of the plant cells resulting in a lower availability of Pi for metabolic functions (Zohlen and Tyler 2004).

Table 5.2 Species used in Chapter 4 for the greenhouse experiment, with the ecological guilds to which they belong according to the IBIF database. P: Pioneers; LLP: Long-lived pioneers; PST: Partial shade tolerant. Responses that species show in ADE are indicated with an X. In the case of mortality some species died in ADE, others on non-ADE and others in both soils (Chapter 4; Fig. 4.1). In the case of nutrient imbalance, some species had less P and N in the leaf tissue of seedlings growing on ADE, other species had less P, and others had less N (Chapter 4: Figs 4.3b, 4.3c, 4.4a, 4.4b).

		Response in Greenhouse Experiment		
Scientific name	**Ecological guild**	**Mortality**	**Nutrient imbalance**	**Not affected**
Albizia niopiodes (Spruce ex Benth.) Burkart	LLP		X (N and P)	
Anadenanthera colubrina (Vell.) Brenan	LLP			X
Enterolobium contortisiliquum (Vell.) Morong	P		X (N and P)	
Erythrina crista-galli L.	P		X (N and P)	
Hymenaea courbaril L.	PST		X (N and P)	
Machaerium villosum Vogel	LLP		X (N)	
Ormosia nobilis Tul	PST			X
Poeppigia procera C. Presl	LLP	X (> on ADE)		
Samanea tubulosa (Benth.) Barneby & J.W. Grimes	PST		X (N and P)	
Schizolobium parahyba (Vell.)S.F. Blake	LLP	X (> on non-ADE)		
Terminalia oblonga (Ruiz & Pav.) Steud.	PST	X (> on ADE)		
Cariniana ianeirensis R. Knuth	PST		X (P)	
Chorisia speciosa A. St.-Hil.	LLP		X (N)	
Guazuma ulmifolia Lam.	LLP	X (= on both soils)		
Cedrela fissilis Vell.	LLP			X
Swietenia macrophylla King	PST		X (P)	
Sapindus saponaria L.	PST			X

Amongst nutrients, nitrogen (N) and phosphorus (P) are the major limiting elements for primary productivity of forests (Vitousek et al. 2010). In the forest of La Chonta, values of available P are relatively high (average 12.03 mg kg^{-1}) compared with a sample of 71 forests across the Amazon basin (Quesada et al. 2010). The results from Chapter 3, in which increases in P and N in the soils did not translate into increases in tree growth suggest that soil pH could modify the availability of those nutrient for the plants (cf.Lambers et al. 2008a) which, in turn, could affect plant distribution. Further studies on tropical tree nutrition and the interactions between soil pH and nutrients in the soils could help to understand these results in depth. There is growing evidence that soil pH is playing an important role in species distribution in the tropics (John et al. 2007, Baldeck et al. 2013)

Implications of soil fertility for plant performance

Ecologist are still in the process of understanding many aspects of plant-soil interactions; i.e. the relationship between nutrients in the soils and in plant tissue (Metali et al. 2015), and this is particularly the case for plant nutrition in tropical forest (Baribault et al. 2012). Information on this topic comes mainly from 'special ecosystems', such as extremely poor soils, P deficient soils, such as the *campos rupestres* in Central Brazil (Oliveira et al. 2015); from poor old soils in the Guyana Shield (Raaimakers and Lambers 1996) and in western Australia (Lambers et al. 2008b). Studies on the association between soil characteristics and tree distributions (John et al. 2007, Jones et al. 2008, Condit et al. 2013) and studies in responses of tree species to soil fertilization allow us to determine for example, how low-wood density species respond more to P additions in the soil than species of high wood density (Baribault et al. 2012). Most ecological studies so far have focused on N and P as the main soil nutrients limiting plant growth and productivity, because they are needed to produce Rubisco and ATP (Lambers et al. 2008a). In plant nutrition and agriculture there is a long tradition on focusing on other limiting elements (Epstein and Bloom 2005, Marschner and Marschner 2012). Now, ecologists also recognize the importance of other elements such as K, Ca, Zn, and B and their interactions for plant nutrition (Watanabe et al. 2007, Wright et al. 2011, Baribault et al. 2012). Likewise, more attention has been given to soil pH on tropical plant distribution, growth and nutrition (Jones et al. 2013, Viani et al. 2014). In temperate zones, plant nutrition studies in relation with calcareous high pH soils have allowed to determine plant strategies that cope successfully or unsuccessfully with P deficiencies (Zohlen and Tyler 2004). However, in tropical forest work on soil pH has mainly been done in acid soils (Viani et al. 2014), mainly because, in general, tropical forest tend to have low values of soil pH. In this sense my thesis can make a modest contribution to the field of plant nutrition. Thus, from this thesis we know that increases in soil pH can be negative for diversity *cf.* ferns (Chapter 2) and that high pH in combination with high Ca can potentially generate nutrient imbalance that negatively affect plant growth (Chapter 4).

Legacies of past inhabitants in the soil and the forest

Legacies are the imprints that humans leave in their environment in the course of their existence. Human legacies are determined by the intact memory (Vogt et al. 1997) of any past modification. Legacies in the soil are persistent changes in chemical and physical characteristics (Vogt et al. 1997) such as the presence of shell middens accumulation in the East coast of the United States, in which the concentration of Ca in the soil and soil pH are remarkable different from surrounding soils (Cook-Patton et al. 2014). Legacies in the forest are the persistent presence of certain plant species, like in the *Maya forest*, in which plant distribution is a reflection of the agroforestry practices of the Maya culture around 1000 yr BP (Ross 2011, Ross et al. 2014); and in relatively young forest that grow on ADE, where there is a higher proportion of domesticated and semi domesticated species (Clement et al. 2003).

The most recent evidence of past human inhabitation in the La Chonta forest is from around 400 year BP (Paz-Rivera and Putz 2009). The possible imprint of this occupation can be inferred from the age of trees and their ecological characteristics. Studies using annual rings have shown that species typical of early to intermediate stages of succession, such as the long-lived pioneer *Sweetia fruticosa*, have a maximum age of around 220 years. Partial-shade tolerant species, such as *Cariniana ianerensis* and *Hura crepitans* have a maximum age of around 170 and 163 years, respectively, and shade tolerant species such as *Ampelocera ruizii*, have a maximum age of around 82 years (sampled in 92 individuals DBH<65 cm) (Vlam et al. 2014). I show in Chapter 3 that tree species composition suggests that this forest is still in a successional stage. I found that large trees (>40 cm stem diameter at 1.3 m height) are represented mainly by long-lived pioneers, the intermediated size trees are represented by partial-shade tolerant species and the smaller trees were mostly represented by shade-tolerant species that showed, on average, the highest tree growth (Chapter 3). Thus, it is possible that openings in the forest made by ancient inhabitants of La Chonta were spread in the area and that the forest is still recovering from these perturbations. Consequently, these patches are still in a late-succesisonal stage. It is also possible, however, that natural process such as transformation from dry forest to humid forest that occurred around 1200 yr BP (Taylor et al. 2010) could be playing a role in this successional composition. I would argue, against the latter because with 100 years of succession, enough time must have passed for the forest to show characteristics of mature stands (*cf.* Saldarriaga *et al.* 1988). A better understanding on how ecosystems change and develop over the long-term, as well as a better knowledge of the ancient inhabitation of La Chonta could help to clarify this aspect.

In summary, in the old-growth forest of La Chonta, legacies of ADE are direct and indirect. Direct legacies include: 1) persistent changes in concentrations of soil nutrients such as Ca and P, and associated changes in soil pH; 2) changes in the abundance of some palms, such as *Astrocarium acculeatum.* Indirect legacies include: 3) changes in species composition of understory species, and the successional composition of the forest through the increase in the concentration of Ca and pH (Figure 5.5).

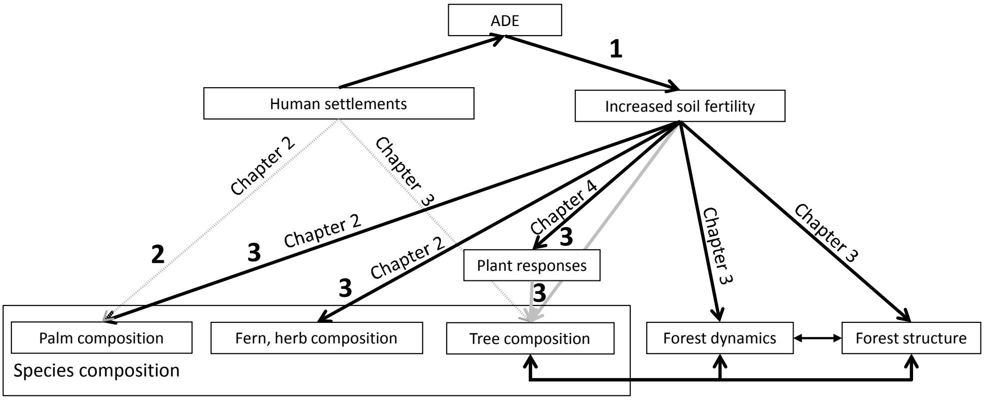

Figure 5.5 Conceptual diagram relating components that explain forest composition and dynamics, evaluated in each chapter, and their relationship with components that represent the legacies of human occupation of La Chonta. Black arrows point out attributes and processes that were directly evaluated in this thesis and for which I found a direct effect of the soils. Solid grey arrows represent attributes for which I found an indirect effect of the soils. Dashed grey arrows represent attributes for which I found a possible effect of human settlements but which I did not evaluate directly. Numbers represent the different legacies of past human inhabitation on the forest of La Chonta: 1. Legacies in the soil; 2. Legacies of 'cultivated' species on current species composition; and 3. Legacies through changes in the soil, in the attributes and processes of the forest.

Recommendations for future research

Results of the present thesis highlight the importance of a better understanding of the effects of soil fertility and soil manipulation on forest dynamics. Nevertheless, far from a solved issue, this thesis opens some new questions that can direct future research in plant and soil interactions, and hopefully bring better understanding of the legacies of the past inhabitants of the Amazonian forest. Some specific future research questions are: First, could tree responses to soil Ca and soil pH explain the distribution patterns of species in the Amazonian forest?; Second, to what extent is the distribution of domesticated or man-used species in the Amazonian region affected or favoured by human induced changes in the soil?

Concluding Remarks

The history of a Bolivian forest, what I refer in this thesis as the southern limit of the Amazon basin, has gone through a long and intense history of human occupation. In this region, the formation of Amazonian Dark Earths probably did not imply soil improvement given the high fertility of the underlying natural soils. Still, small changes in the soil as a consequence of human inhabitation, have left long lasting impacts on the diversity of ferns and the composition of plants in the forest. Thus, through subtle changes in the soil, humans can have long lasting impacts on the forest.

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APPENDIX

Characterization of soils at La Chonta

This appendix contains data on the characterization of the soils of the forestry concession of La Chonta, Guarayos province, Bolivia (15º47'S, 62º55'W).

Methodology

Data for the characterization of the soils were obtained during a field trip made to La Chonta from January 31st to February 3rd of 2013. The weather during the trip was overcast intermixed with heavy rains. I visited two of the permanent plots that are part of the Long-term Silvicultural Research Program (LTSRP) (see (Peña-Claros et al. 2008), and chapter 4 for details). The first plot, Block 2 Intensive, is located at 2.64 km (north-east) from the camping facilities that IBIF has in the concession; the second plot, located at 2 Km (north-east) from the camping, is denominated Block 2 Normal. In previous soil surveys made from September to December of 2011 on the 12 ha permanent plots, we determined soil color and presence of ceramics on grids of 50x50 m (Klomberg 2012). Based on the previous description of soil color and records of ceramics in the plots, we established a transect of around 275 m length in each plot. Using an auger, I collected soil samples at depth intervals of 20 cm, every 75 m, but I shortened this distance (25 to 50 m) when I were close to points that previously were recorded as having ceramics (see map in Fig. A1a). Besides, I collected an extra sample in the point (250,100) where I noticed changes in the terrain topography, so I could get a better description of soil variability (see map in Fig. A1a). Because of heavy rains during the trip, we could only take three samples from plot Block 2 Normal from a site that had dark soils and records of ceramics (see map in Fig. A1b). Additionally, we took a sample (P_{12}) from a site that was previously described by Paz-Rivera and Putz (2009) as having abundant ceramics and containing anthropic soil horizon. To establish the profiles, I removed the litter layer from the top soil and I dug a one-meter depth pit using a Dutch auger; thereafter, I divided the profiles at intervals of depth of 20 cm (0-20; 20-40; 40-60; 60-80; 80-100 cm). In the field notes I recorded the depth of the limit of horizons in the soil, based in change in color and texture (done by finger methods in the field). I collected a sample of each depth and I saved it for further analysis in Santa Cruz, Bolivia.

Once in Santa Cruz, I characterized the physical and chemical properties of the soil at each depth on all the profiles. I characterized soil color using the Munsell Color guide over a wet sample. Soil texture was characterized by taking one table spoon of soil and adding water until sticky consistency was reached; then the soil was rolled in a ball and texture was determined following the method in McGarry (2006). I assessed soil pH and magnetic susceptibility for all samples by taking 25 mg of soil per sample and combining it with 10 ml of distilled water; I then measured pH with a Thermo Fisher Scientific Orion start meter (Beverly, MA, USA). Finally, the soil samples were analyzed with additional chemical and physical parameters at the Centro de Investigación Agrícola Tropical (CIAT) in Santa Cruz, Bolivia. Concentrations of N, available P (Olsen), exchangeable Ca^{2+}, Mg^{2+}, K^{+} and Al^{+3}; pH in water (1:2,5 v/v); soil carbon (organic matter content -OM-); total exchangeable bases (TEB); cation exchange capacity (CEC); base saturation; and particle size distribution for sand, silt, and clay were determined (for detailed methods on chemical and physical characterization of soils, see Appendix of Chapter 2).

Location of profiles in the permanent plots

The location of the auger profiles is showed in Fig. A1a. Note that when I approached a point where ceramic had been recorded, I shortened the distance between points. Fig. A1b, shows three profiles that were taken in plot Block 2 Normal; Figs. A3 and A4 show pictures of the soil profiles, where soil color can be appreciated; finally, Figs A5 and A6 show some of the chemical and textural variables of the soil of each profile.

Soil classification

In general, some morphological detail is necessary to classify soils; moreover, the profiles should be analyzed based on horizons and not by “artificial” layers. Nevertheless, I am confident that the data of the physical and chemical analyses presented here, together with the field notes, allowed us to ascertain the soil classes that I found in La Chonta.

Soils at La Chonta are mostly Cambisols, although there are also Histosols, Gleysols, Leptosols/Regosols and Anthrosols. Presence of other soils typical of the region, such as Acrisols, Lixisols and Luvisols, is improbable because there is not a gradient in clay content (*argic* horizon), which is necessary to characterize these soil classes. I also ruled out Nitisols because there was not enough clay content (*nitic* horizon) that characterizes this class of soil. I can also discard the presence of Ferralsols because soils at La Chonta present a ratio silt:clay ratio higher than that of soils that are highly weathered like Ferralsols. Likewise, the amount of some primary minerals, visible in soil samples, is high in La Chonta soils making the presence of Ferralsols less likely. Finally, base saturation in soils of La Chonta is high enough that I can discard the presence of Alilsols (Gardi et al. 2014).

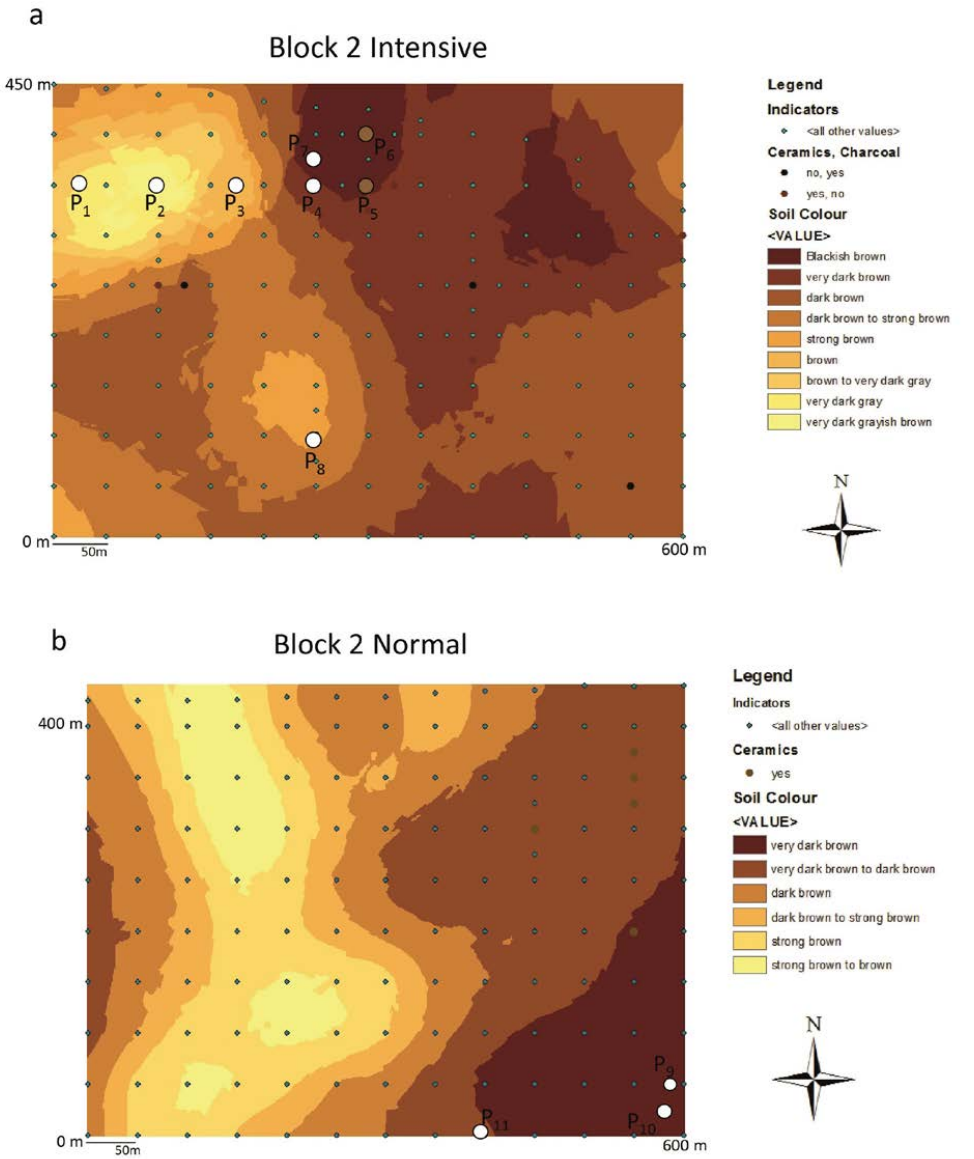

Figure A.1 Maps from two permanent plots at forestry concession La Chonta, Guarayos Province, Bolivia. Color of the maps was obtained using ordinary Kriging function with a spherical semi-variogram model within the Interpolation box of the Spatial Analyst Tool in ArcMAP sotware based in field soil color descriptions using the Munsell Chart Color guide (See, Klomberg 2012 for details). Presence of ceramics and charcoal is shown. **a**) location of profiles where soils were collected (Profiles P) in the plot Block 2 Intensive. Notice that Profile 5 (P_5) and profile 6 (P_6) had ceramics. **b**) location of soil collection profiles in the plot Block 2 Normal.

Cambisols have, in general, incipient formation indicated by a *cambic* (change) horizon with variation in color, structure and clay content. These soils are generally young, not necessarily very fertile, but they can contain minerals from bed rock. In La Chonta they seem to be present in plains and small slopes (Fig. A2). Histosols and Gleysols are soils present in lower areas in the landscape and usually they are associated with ground water, or low soil drainage. In La Chonta they could be present in low areas (Fig. A2) (Profile 3). Leptosols are thin soils, not very deep, with coarse material and presence of gravel very close to the soil surface; they are found in sites with some dissected topography; therefore, soil fertility is variable and depending of the bed rock, they can have high base saturation. In La Chonta some of these soils are present in the top of the hills, normally associated to rock in the surface (Fig. A2) (Profile 8). Finally, Anthrosols are present in La Chonta, indicated by the presence an horizon with dark soil color (munsell 7.5YR 2.5/1) and remains of artefacts, such as pieces of ceramics or human generated charcoal; they present high concentration of P and high total base concentration (Profile 6, 9, 10, 12). The profile P_{12} is an example of a Anthrosol with enrichment of P, high organic matter content until 60 cm, very dark color and with ceramic bricks (Fig. A7). The values of pH are high, possibly indicating anthropic modification (Woods, 2009). A previous study in La Chonta found that *ca.* 20% of surveyed area of 216 ha had Anthrosols, or '*terra preta*' (Paz-Rivera and Putz 2009), indicating that these soils are relatively common. A strict classification of the soil in low levels (as *pretic*, *hortic*, *plaggic* Anthrosols) was not the objective of this study and some additional information that I did not collect is needed for this task.

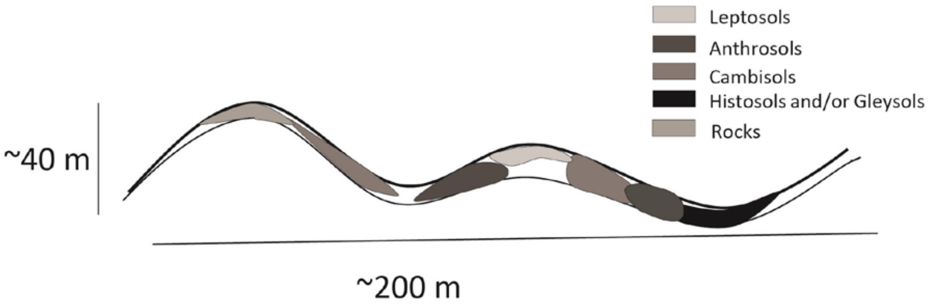

Figure A.2 General scheme of distribution of soils classes in the landscape of the forestry concession La Chonta, Guarayos. Province, Bolivia. Based on a scratch scheme made during the field trip (Wenceslau Texeira). Scales are approximated.

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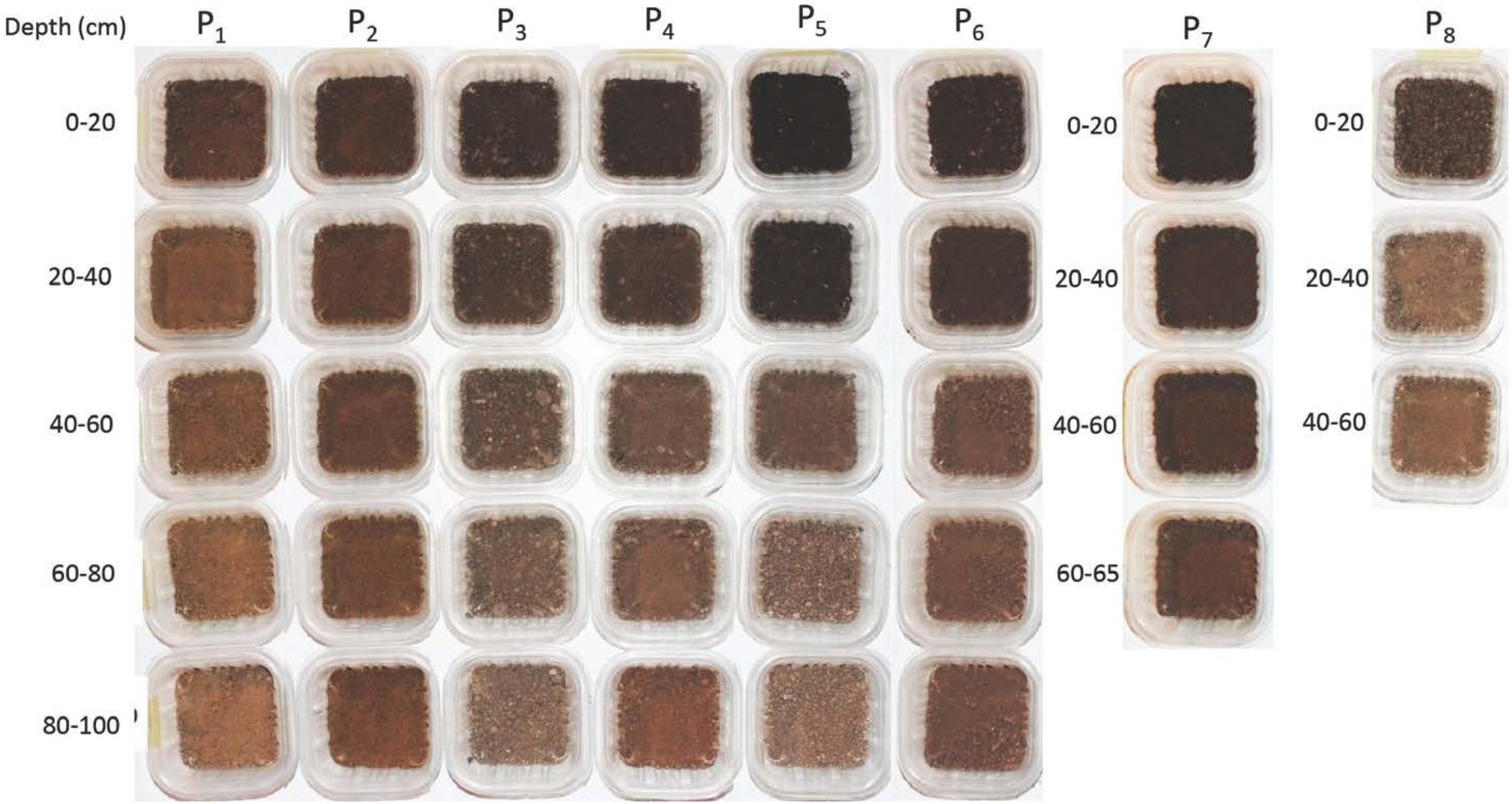

Figure A.3 Photograph of soil samples collected at different layers from plot Block 2 Intensive from the forestry concession La Chonta, Guarayos Province, Bolivia. Numbers on the left side represent the depth of the sample (in cm). and the label of each profile is indicated at the top. Notice that P_7 and P_8 are less deep than the rest of the profiles. Photo: Daniel Alarcon & Carmen Mateu.

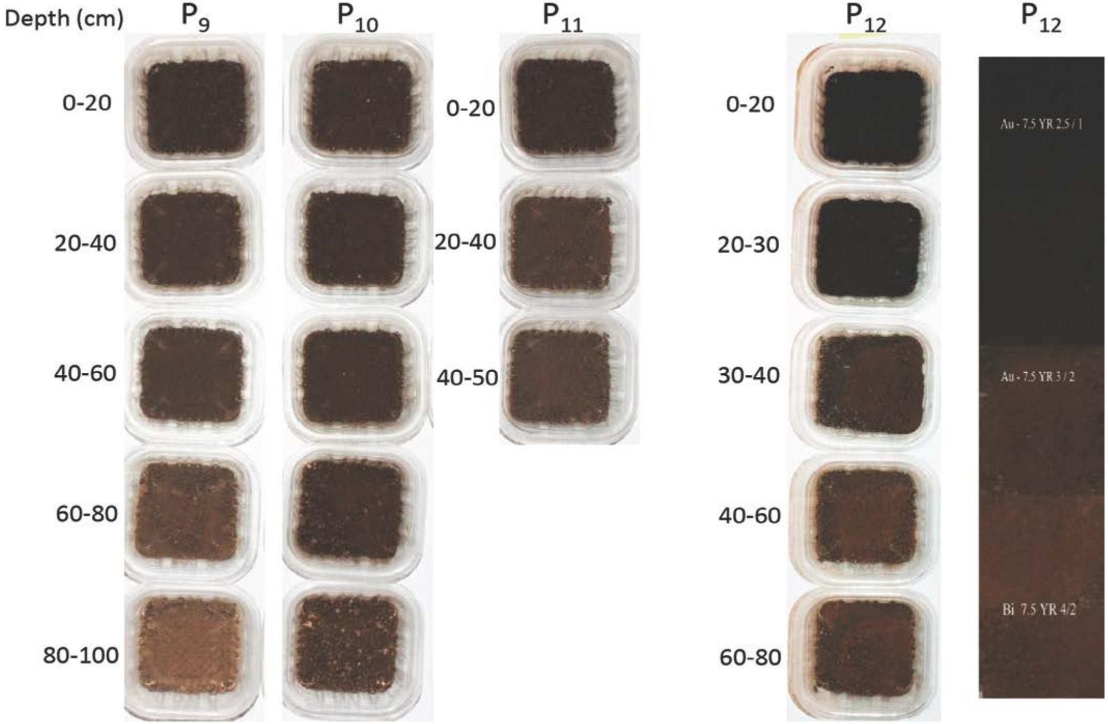

Figure A.4 Soil profiles from Plot Block 2 Normal (P_9 to P_{11}) and from the site Chonta 7 (P_{12}) at the forestry concession La Chonta, Guarayos Province, Bolivia. Numbers on the left side represent the depth of the sample in cm. and the label of each profile is in the top. Notice that P_{11} is less deep than the other two profiles from Block 2 Normal. Profile 12 also shows differentiation in horizons (Wenceslau Texeira). Pieces of ceramics were found in this profile in a depth of 0-30 cm. Photo: Daniel Alarcon & Carmen Mateu.

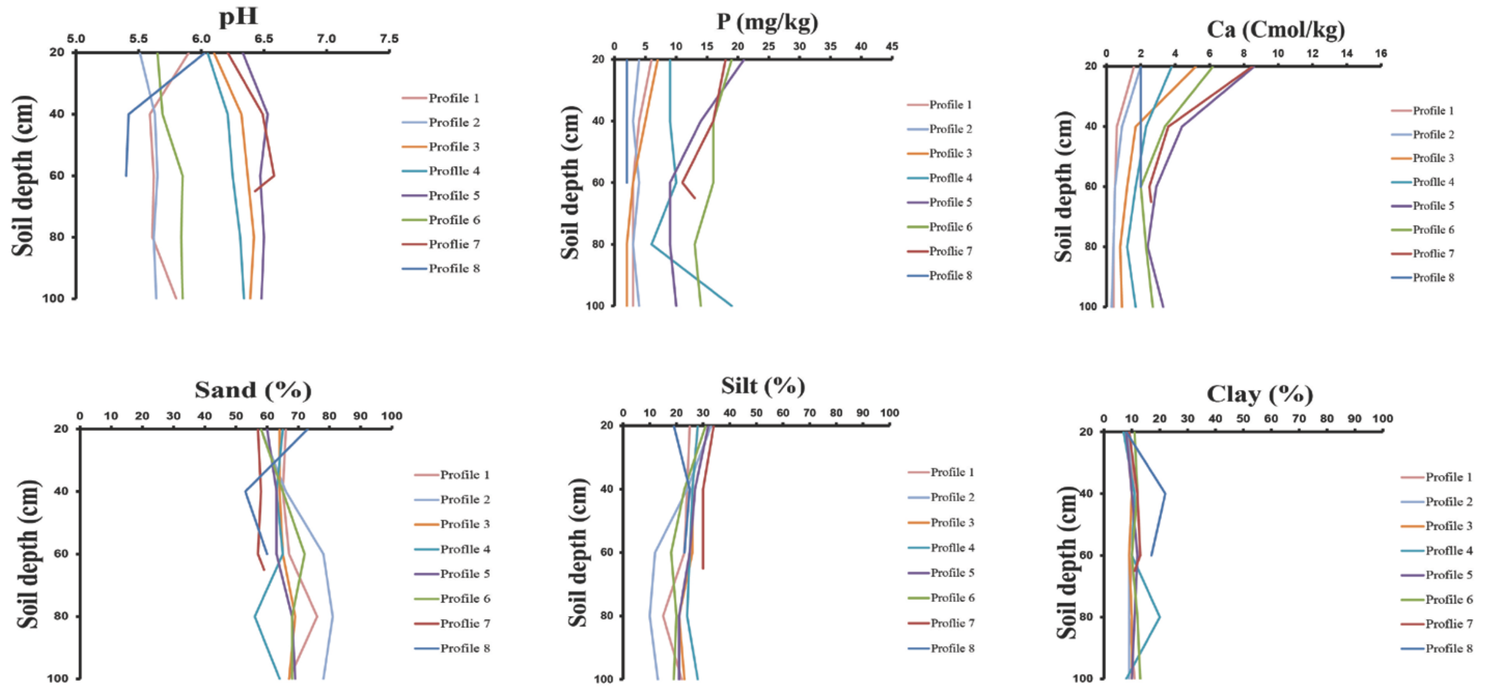

Figure A.5 Chemical and texture variables in the different depth intervals in the profiles collected in the permanent plot Block 2 Intensive of the forestry concession of La Chonta, Guarayos province, Bolivia. The upper panel shows chemical variables: pH, P and Ca; and the lower panel shows texture variables: percentage of sand, clay and silt.

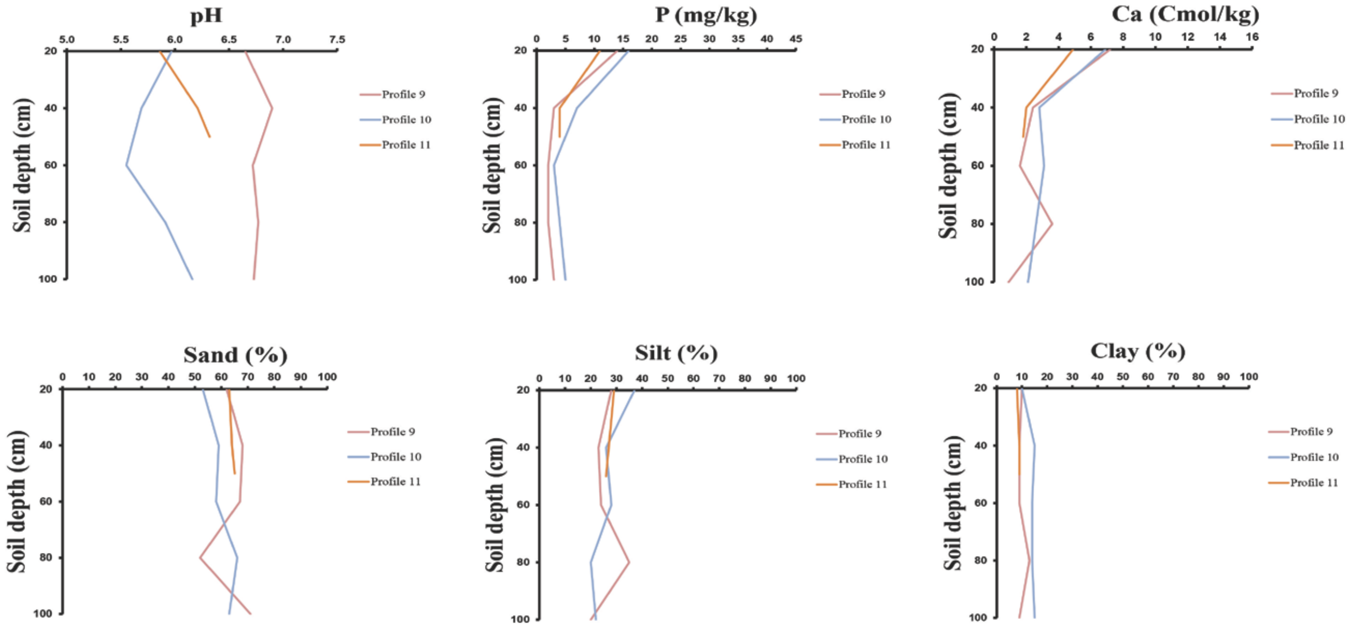

Figure A.6 Chemical and texture variables in the different depth intervals in the profiles collected in the permanent plot Block 2 Normal of the forestry concession of La Chonta, Guarayos province, Bolivia. The upper panel shows chemical variables: pH, P and Ca; and he lower panel shows texture variables: percentage of sand, clay and silt.

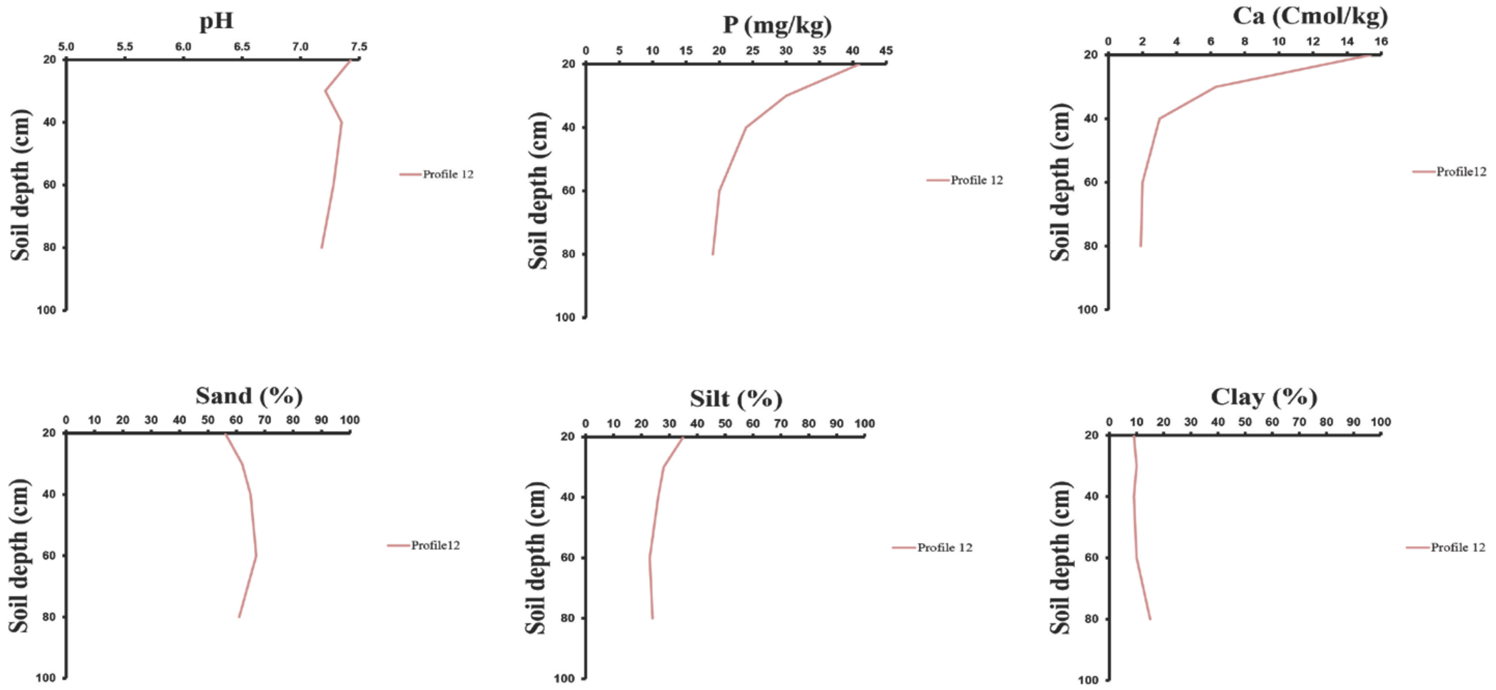

Figure A7 Chemical and texture variables in the different depth intervals in the profile collected in a site that previously described as "*Terra Preta*" (Paz-Rivera and Putz, 2009) in the forestry concession of La Chonta, Guarayos province, Bolivia. In the field survey, pieces of ceramics were found associated to this profile. The upper panel shows chemical variables: pH, P and Ca; and the lower panel shows texture variables: percentage of sand, clay and silt.

Table A.. Physical and physicochemical variables of 12 soil profiles collected in two permanent plots at La Chonta forestry concession, in Guarayos province, Bolivia. Electric conductivity units are µS cm^{-1}. B2 I = Block 2 Intensive; B2 N = Block 2 Normal and Chonta 7 is an Amazonian Dark Earths site (Paz-Rivera and Putz, 2009)

Depth (cm)	Electric Conductivity (µS cm^{-1})	Soil color	Soil texture	Sand (%)	Silt (%)	Clay (%)
Site B2 I -Profile 1- Coordinates (UTM) 525768, 8265150.3						
0-20	87.9	7.5 YR 3/4	silty loam	66	25	9
20-40	31	7.5 YR 4/4	sandy loam	65	24	11
40-60	18.01	7.5 YR 4/4	sandy loam	67	23	10
60-80	17.26	10 YR 5/4	sandy loam	76	15	9
80-100	19.05	10 YR 5/4	sandy loam	67	22	11
Site B2 I -Profile 2- Coordinates (UTM) 525843, 8265150.3						
0-20	85.73	7.5 YR 3/3	silty loam	58	33	9
20-40	29.1	7.5 YR 3/4	silty loam	66	24	10
40-60	17.78	7.5 YR 4/4	sandy loam	78	12	9
60-80	16.34	7.5 YR 4/6	sandy loam	81	10	9
80-100	11.8	7.5 YR 4/6	sandy loam	78	13	9
Site B2 I -Profile 3- Coordinates (UTM) 525918, 8265150.3						
0-20	175.67	7,5 YR 2,5/2	loam	64	28	8
20-40	79.97	7,5 YR 2,5/3	loam	64	26	10
40-60	68.67	7,5 YR 3/3	silty loam	65	26	9
60-80	50.23	7.5 YR 3/4	sandy loam	69	21	10
80-100	53.57	7,5 YR 4/4	sandy	67	23	10
Site B2 I -Profile 4- Coordinates (UTM) 525988, 8265149						
0-20	102.23	7,5 YR 2,5/2	silty loam	65	28	7
20-40	108.73	7,5 YR 2,5/2	silty loam	63	26	11
40-60	85	7,5 YR 2,5/3	sandy loam	65	25	10
60-80	86.37	7,5 YR 3/4	sandy loam	56	24	20
80-100	51	7,5 YR 4/6	clayley loamy	64	28	8
Site B2 I -Profile 5 - Coordinates (UTM) 526045, 8265145						
0-20	140.37	7,5 YR 2,5/1	silty loam	60	32	8
20-40	64.67	7,5 YR 2,5/1	silty loam	63	27	10
40-60	38.57	7,5 YR 2,5/2	silty loam	63	25	12
60-80	31.3	7,5 YR 4/4	sandy loam	68	21	11
80-100	28.94	7,5 YR 4/4 4/3	loam	69	21	10

Table A.1 (Continued)

Depth (cm)	Electric Conductivity (μS cm^{-1})	Soil color	Soil texture	Sand (%)	Silt (%)	Clay (%)
Site B2 I - Profile 6 - Coordinates (UTM) 526039, 8265200						
0-20	57.63	7,5 YR 2,5/1	silty loam	58	31	11
20-40	24.44	7,5 YR 2,5/2	silty loam	65	23	12
40-60	18.19	7,5 YR 2,5/3	silty loam	72	18	10
60-80	15.8	7,5 YR 3/4	sandy loam	68	20	12
80-100	18.8	7,5 YR 3/4	silty loam	68	19	13
Site B2 I -Profile 7- Coordinates (UTM) 525978, 8265169						
0-20	155.23	7,5 YR 2,5/2	loam	57	34	9
20-40	33.57	7,5 YR 2,5/2	loam	58	30	12
40-60	16.29	7,5 YR 2,5/2	loam	57	30	13
60-65	24.97	7,5 YR 3/4	clayley loamy	59	30	11
Site B2 I Profile 8 Coordinates (UTM) 525984, 826846						
0-20	88.83	7,5 YR 3/2	heavy clay	73	19	8
20-40	13.52	7,5 YR 4/3	fine clay	53	25	22
40-60	11.69	7,5 YR 3/4	clayley loamy	60	23	17
Site B2 N -Profile 9- Coordinates (UTM) 525227, 8265018						
0-20	222.9	7,5 YR 2,5/2	loam	62	28	10
20-40	41.67	7,5 YR 2,5/2	silty loam	68	23	9
40-60	16.39	7,5 YR 3/3	silty loam	67	24	9
60-80	12.03	7,5 YR 3/3	silty loam	52	35	13
80-100	15.83	7,5 YR 3/4	silty loam	71	20	9
Site B2 N -Profile 10- Coordinates (UTM) 525213, 8264991						
0-20	149.75	10 YR 2/2	loam	53	37	10
20-40	18.48	10 YR 2/2	loam	59	26	15
40-60	29.45	10 YR 3/2	fine clay	58	28	14
60-80	14.08	10 YR 3/2	silty loam	66	20	14
80-100	13.15	10 YR 3/3	silty loam	63	22	15
Site B2 N -Profile 11- Coordinates (UTM) 525033, 8264976						
0-20	101.93	7,5 YR 3/2	loam	63	29	8
20-40	40.23	7,5 YR 3/2	silty loam	64	27	9
40-50	38.6	7,5 YR 3/3	sandy loam	65	26	9

Table A.1 (Continued)

Depth (cm)	Electric Conductivity (µS cm^{-1})	Soil color	Soil texture	Sand (%)	Silt (%)	Clay (%)
Chonta 7 -Profile 12- Coordinates (UTM) 524520, 8263152						
0-20	216.77	7,5 YR 2,5/1	loam	56	35	9
20-30	79.1	7,5 YR 2,5/1	silty loam	62	28	10
30-40	59.2	7,5 YR 3/2	silty loam	65	26	9
40-60	43	7,5 YR 3/2	silty loam	67	23	10
60-80	39.15	7,5 YR 4/2	silty loam	61	24	15

Table A.2 Physical and physicochemical variables of 12 soil profiles collected in two permanent plots at La Chonta forestry concession, in Guarayos province, Bolivia. TEB = Total exchangeable bases, and CEC = cation exchange capacity. Units of Ca, Mg, Na, K, TEB, CEC, and Al are cmol kg^{-1}. Units of P are mg kg^{-1}. B2 I = Block 2 Intensive; B2 N = Block 2 Normal and Chonta 7 is an Amazonian Dark Earths site (Paz-Rivera and Putz, 2009)

Depth (cm)	pH (H_2O)	Ca	Mg	Na	K	TEB	CEC	Sat bas (%)	Acidity	Al	P	OM (%)
Site B2 I -Profile 1- Coordinates (UTM) 525768, 8265150.3												
0-20	5.9	1.6	0.8	0.03	0.1	2.5	2.7	94	0.2	0	6	1.9
20-40	5.59	0.6	0.7	0.03	0.07	1.4	2.3	60	0.9	0	4	1.3
40-60	5.62	0.5	0.6	0.04	0.05	1.2	2.2	55	1	0	3	0.4
60-80	5.61	0.4	0.5	0.04	0.04	1	2	50	1	0	3	0.3
80-100	5.8	0.4	1	0.05	0.04	1.5	2.4	61	0.9	0	3	0.2
Site B2 I -Profile 2- Coordinates (UTM) 525843, 8265150.3												
0-20	5.51	2	1.1	0.05	0.11	3.3	3.4	95	0.2	0	4	2.2
20-40	5.63	0.9	1.7	0.04	0.05	2.7	2.9	93	0.2	0	3	1
40-60	5.65	0.5	0.4	0.03	0.09	1	2	52	1	0	4	0.4
60-80	5.62	0.4	0.4	0.03	0.03	0.9	1.8	47	1	0	3	0.3
80-100	5.64	0.3	0.6	0.04	0.03	1	2.1	46	1.2	1	4	0.3
Site B2 I -Profile 3- Coordinates (UTM) 525918, 8265150.3												
0-20	6.1	5.2	1.2	0.05	0.19	6.6	6.6	100	0	0	7	2.5
20-40	6.32	1.7	0.9	0.04	0.1	2.7	2.7	100	0	0	5	0.1
40-60	6.37	1.2	0.9	0.04	0.12	2.3	2.3	100	0	0	3	0.9
60-80	6.42	0.8	0.8	0.05	0.11	1.8	1.8	100	0	0	2	0.6
80-100	6.39	0.9	0.9	0.05	0.16	2	2	100	0	0	2	0.5

Table A.2 (Continued)

Depth (cm)	pH (H_2O)	Ca	Mg	Na	K	TEB	CEC	Sat bas (%)	Acidity	Al	P	OM (%)
Site B2 I -Profile 4- Coordinates (UTM) 525988, 8265149												
0-20	6.05	3.8	0.9	0.05	0.19	4.9	5.1	98	0.1	0	9	3
20-40	6.21	2.3	0.8	0.04	0.19	3.3	3.3	100	0	0	9	1.9
40-60	6.25	1.7	0.8	0.07	0.12	2.7	2.7	100	0	0	10	0.9
60-80	6.31	1.2	0.8	0.05	0.09	2.1	2.1	100	0	0	6	0.7
80-100	6.34	1.7	1.5	0.05	0.19	3.4	3.4	100	0	0	19	0.6
Site B2 I - Profile 5- Coordinates (UTM) 526045, 8265145												
0-20	6.33	8.6	1.7	0.04	0.28	10.6	10.6	100	0	0	21	3.4
20-40	6.53	4.4	1.6	0.04	0.15	6.2	6.2	100	0	0	14	2.5
40-60	6.47	2.9	2.4	0.05	0.26	5.6	5.6	100	0	0	9	1
60-80	6.5	2.4	1.7	0.05	0.26	4.4	4.4	100	0	0	9	0.4
80-100	6.48	3.3	1.8	0.05	0.25	5.4	5.4	100	0	0	10	0.4
Site B2 I -Profile 6- Coordinates (UTM) 526039, 8265200												
0-20	5.65	6.2	1.9	0.04	0.34	8.5	8.6	98	0.2	0	19	3.2
20-40	5.69	3.4	0.9	0.05	0.17	4.5	4.7	96	0.2	0	16	2.6
40-60	5.85	2	0.6	0.07	0.13	2.8	2.9	95	0.1	0	16	1.3
60-80	5.84	2.3	0.7	0.07	0.14	3.2	3.4	95	0.2	0	13	1
80-100	5.85	2.7	0.8	0.07	0.14	3.7	3.9	96	0.1	0	14	0.9

Table A.2 (Continued)

Depth (cm)	pH (H_2O)	Ca	Mg	Na	K	TEB	CEC	Sat bas (%)	Acidity	Al	P	OM (%)
Site B2 I -Profile 7- Coordinates (UTM) 525978, 8265169												
0-20	6.21	8.5	1.3	0.09	0.36	10.3	10.3	100	0	0	18	2.9
20-40	6.49	3.6	0.7	0.05	0.22	4.6	4.7	98	0.1	0	16	1.3
40-60	6.58	2.5	0.3	0.08	0.16	3	3.2	96	0.1	0	11	0.9
60-65	6.43	2.6	0.3	0.04	0.15	3.1	3.2	96	0.1	0	13	0.6
Site B2 I -Profile 8- Coordinates (UTM) 525984, 826846												
0-20	6.03	1.2	0.5	0.05	0.09	1.8	2	93	0.1	0	2	0.7
20-40	5.42	0.9	0.4	0.09	0.09	1.5	2.8	52	1.4	1.1	2	0.7
40-60	5.4	0.6	0.4	0.11	0.09	1.2	2.4	51	1.2	0.9	2	0.6
Site B2 N -Profile 9- Coordinates (UTM) 525227, 8265018												
0-20	6.65	7.2	1.7	0.04	0.19	9.1	9.1	100	0	0	14	3.2
20-40	6.9	2.4	0.5	0.03	0.13	3.1	3.1	100	0	0	3	1.7
40-60	6.72	1.6	0.4	0.03	0.1	2.1	2.3	95	0.1	0	2	1.1
60-80	6.77	3.6	1	0.05	0.24	4.9	5.1	97	0.2	0	2	0.5
80-100	6.73	0.9	0.5	0.04	0.1	1.5	1.6	94	0.1	0	3	0.3

Table A.2 (Continued)

Depth (cm)	pH (H_2O)	Ca	Mg	Na	K	TEB	CEC	Sat bas (%)	Acidity	Al	P	OM (%)
Site B2 N -Profile 10- Coordinates (UTM) 525213, 8264991												
0-20	5.97	6.9	1.7	0.04	0.18	8.8	8.8	100	0	0	16	2.9
20-40	5.69	2.8	0.4	0.04	0.11	3.4	3.4	98	0.1	0	7	1.2
40-60	5.55	3.1	0.7	0.04	0.13	4	4.1	98	0.1	0	3	1.4
60-80	5.91	2.6	0.3	0.04	0.11	3.1	3.2	96	0.1	0	4	0.9
80-100	6.16	2.1	0.4	0.05	0.12	2.7	2.8	95	0.1	0	5	0.8
Site B2 N -Profile 11- Coordinates (UTM) 525033, 8264976												
0-20	5.86	4.9	1	0.04	0.26	6.2	6.2	100	0	0	11	2.7
20-40	6.21	2	0.6	0.05	0.12	2.8	2.8	100	0	0	4	1.4
40-50	6.32	1.8	0.5	0.04	0.12	2.5	2.5	100	0	0	4	1.4
Chonta 7 -Profile 12- Coordinates (UTM) 524520, 8263152												
0-20	7.43	15.4	0.8	0.04	0.43	16.7	16.7	100	0	0	41	3.1
20-30	7.21	6.3	0.4	0.04	0.38	7.1	7.1	100	0	0	30	1.8
30-40	7.35	3	0.2	0.03	0.24	3.5	3.5	100	0	0	24	1.4
40-60	7.28	2	0.2	0.03	0.15	2.4	2.4	100	0	0	20	0.8
60-80	7.18	1.9	0.2	0.03	0.13	2.3	2.3	100	0	0	19	0.7

Summary

Amazonian forest is seen as the archetype of pristine forests, untouched by humans, but this romantic view is far from reality. In recent years, there is increasing evidence of long and extensive landscape modification by humans. Processes of permanent inhabitation, expansion and retreat of human populations have not always been obvious in those ecosystems, leaving sometimes weak and overlooked imprints in the landscape. An example of one of these inconspicuous alterations are the modifications in the soil known as Amazonian Dark Earths (ADE) or '*terra preta*' (black earth in Portuguese), which are the product of the accumulation of residuals from permanent or semi-permanent human inhabitation. They are named after the black color of the soils, which is a consequence of the accumulation of charcoal pieces and organic matter in the soil. These soils also contain higher levels of phosphorous, calcium (mainly originated from bone residuals), and nitrogen that increase fertility of the naturally poor soils, thus favouring agricultural practices. Amazonian Dark Earths are distributed in Brazil, Bolivia, Colombia, and Peru, and it is estimated that they could occupy 3% of the area of the Amazon basin.

With the decrease in human population in the Americas after the encounter with European colonists, sites where ADE had been formed were abandoned and the vegetation recovered. So far, the effects of ADE on old growth forest had not been widely examined and we are just starting to understand the consequences of past human inhabitation on forest composition and structure. In this thesis, I evaluated the effects of ADE on the forest that has re-grown after abandonment by indigenous people in the La Chonta forest, situated at the southern edge of the Amazon basin, in Bolivia. First, I assessed the magnitude of the changes in the soil as a consequence of human occupation. For that, I did a detailed soil survey on 12 plots of ~27-ha each, and I evaluate the differences between ADE and non-ADE soils. Then, I studied how soil changes affected plant species composition in the understory (Chapter 2), forest structure and forest dynamics (Chapter 3), and finally I determined how seedlings of tree species respond to anthropogenic changes in soil properties (Chapter 4).

Detailed information on soil characteristics and its heterogeneity in the landscape is needed to evaluate the effects of soil on the vegetation. This was even more important for my study site, as soil heterogeneity can be increased by the presence of ADE. Therefore, I did detailed soil surveys (Appendix, Chapter 2, Chapter 3) that allowed me to understand the relationship between past human occupation and alteration in the concentration of soil nutrients. I found that natural soils in the southern Amazonian forest are more fertile than their Central and Eastern Amazon counterparts. Past human presence in the area resulted in soil enrichment, due to increases in the concentration of phosphorus, calcium, potassium, and increases in soil pH. Thus, with this information I could test specific hypothesis about the effects of soil fertility on the vegetation that occurs in these sites.

In the Amazonian forest in general, soil characteristics influences the composition of understory angiosperm herbs, ferns and palm species. Thus, increases in soil fertility in ADE

could affect the distribution of understory angiosperm herbs, ferns and palm species. In Chapter 2, I evaluated the effect of ADE on composition, richness and abundance of understory species (ferns, angiosperm herbs, and palms). First, I did a detailed soil survey that allowed me to determine sites in the forest with higher nutrients and with evidence of past human occupation (i.e., presence of ceramics). Then, I correlated soil variables associated with ADE, such as Ca, P, and soil pH, with species composition, richness and abundance. I found that the presence of ADE created a gradient in soil nutrients and pH, which changed the composition of understory species, especially of ferns and palms. Additionally, the higher nutrient concentration and the more neutral pH on ADE soils were associated with a decrease in the richness of fern species. I therefore conclude that the current composition of the understory community in La Chonta is a reflection of past human modification of the soil.

Heterogeneity of soil fertility across the Amazonian region arises from geological history and geomorphological processes. This heterogeneity drives forest structure and forest dynamics across the Amazon region, but at a local scale the role of soils on forest dynamics is not well understood. The study of Amazonian Dark Earths (ADE) opens an opportunity to test how increases in soil fertility could affect forest structure and dynamics at local scales. In chapter 3, I evaluated the effect of ADE on forest attributes, such standing basal area, tree liana infestation and successional composition, defined by the relative presence of pioneers, long-lived pioneers, partial shade tolerant and shade tolerant species in the forest. I also evaluated the effect of ADE on individual components of forest dynamics: basal area growth, recruitment, and mortality. Surprisingly, I found that these fertile ADE affected only few forest attributes and components of forest dynamics. Soil pH was one of the edaphic variables that significantly explained forest structure and dynamics. A higher soil pH increased recruitment of intermediate-sized trees (with stem diameter between 20 and 40 cm) and decreased mortality of large trees (stem diameter > 40 cm). The most important effect of pH, however, was on initial basal area and successional composition, which directly affected growth in basal area of intermediate-sized trees. In conclusion, I found that in this southern Amazonian forest, other environmental factors, such as light availability and liana infestation are stronger drivers of forest dynamics than ADE or soils in general.

Increases in soil nutrients can drive plant responses promoting higher growth rates and lower mortality. Plants respond to soil nutrient availability through a suite of traits, by adjusting their biomass allocation patterns, morphology, tissue chemistry and physiology, which allow them successful establishment and regeneration. The higher amount of nutrients found on ADE compared to natural soils could improve the growth of tropical tree species. In chapter 4, I studied the effect of ADE on seedling growth, morphology and physiology in a greenhouse experiment with seedlings of 17 tree species from La Chonta. I found that soil type affected 10 out of 24 evaluated seedling traits. Seedlings did not invest more in roots in non-ADE (to take up scarce soil resources) but they invested in leaves and leaf area in ADE (to enhance light capture), although this did not lead to faster growth rate. Tree species responded differently to an increase in soil Ca concentration, which was 2.4 times higher in ADE than in non-ADE soils. Some species seemed to suffer from Ca toxicity as indicated by higher seedling mortality on ADE; others suffered from nutrient imbalance as observed from changes in nutrient stoichiometry in the leaves; whereas other species increased their leaf Ca, P and N concentrations in ADE. Only for this latter group of nutrient accumulators, there was a positive relationship between leaf Ca concentration and the growth rates of seedlings. Contrary to expectations, ADE did not lead to increased seedling growth. The ability of plants to colonize patches of ADE might depend on plant responses to increased soil Ca and their capacity to regulate internal tissue calcium to balance nutrition.

In summary, in this southern Amazon forest the increased soil nutrient concentrations are a legacy of the humans that inhabited the area. This nutrient addition caused changes in understory species composition and decreased fern species richness and had modest effects on forest structure and dynamics. Increases in nutrients, specifically Ca, can cause positive and negative responses of tree species, resulting in potentially long term effects on the tree species composition of the forest.

Samenvatting

Het Amazonewoud wordt vaak gezien als hét voorbeeld van een ongerept bos, onaangetast door menselijke beschaving. Maar dit romantische beeld klopt niet. De afgelopen jaren is het bewijs toegenomen dat mensen dit landschap intensief beïnvloed hebben gedurende een lange periode in het verleden. De gevolgen van permanente bewoning en de toe- en afname van menselijke bewoning hebben meestal slechts vage sporen achtergelaten in dit landschap, die gemakkelijk over het hoofd kunnen worden gezien. Een voorbeeld van deze onopvallende sporen zijn de bodems die bekend staan als 'Amazonian Dark Earths'; (ADE), '*terra preta*'. Deze bodems zijn het gevolg van een opeenstapeling van resten van de permanente en semipermanente bewoning door mensen. De bodems danken hun naam aan hun zwarte kleur, die het gevolg is van de aanwezigheid van houtskoolstukjes en organisch materiaal. Ze bevatten ook een hoge concentratie fosfor, calcium (voornamelijk afkomstig uit botfragmenten) en stikstof. Door aanwezigheid van deze nutriënten wordt plaatselijk de bodemvruchtbaarheid verhoogd in de van nature arme bodems in de Amazonebekken en daardoor kan de landbouwopbrengst hoger zijn. Amazonian Dark Earths bodems worden gevonden in Brazilië, Bolivia, Colombia en Peru en bedekken naar schatting 3% van het oppervlak van het Amazonebekken.

Na de ineenstorting van menselijke populaties op het Amerikaanse continent, na de komst van Europese kolonisten, zijn de plaatsen waar ADE gevormd werd verlaten en weer overgroeid met vegetatie. Tot op heden zijn de effecten van ADE op volgroeide bossen nog nooit uitgebreid onderzocht en we beginnen pas recent te begrijpen hoe menselijke bewoning in het verleden de compositie en structuur van bossen in het heden heeft bepaald. In deze thesis heb ik onderzocht hoe ADE het bos heeft beïnvloed dat is terug gegroeid na de verdwijning van inheemse bewoners. Ik heb dit gedaan in het bos 'La Chonta', dat ligt in het zuidelijke grensgebied van het Amazonebekken in Bolivia. In dit gebied heb ik allereest onderzocht hoe ADE bodems en gewone bodems (niet-ADE bodems) verschillen in hun fysische en chemische karakteristieken. Hiervoor heb ik de bodem in 12 percelen van elk ~27 hectare in kaart gebracht. Vervolgens heb ik bestudeerd hoe veranderingen in de bodem effect hebben op de samenstelling van planten in de onderlaag van het bos (hoofdstuk 2), de structuur en dynamiek van het bos (hoofdstuk 3) en tenslotte, hoe zaailingen van bomen reageren op antropogene veranderingen van bodemeigenschappen (hoofdstuk 4).

De beschikbaarheid van gedetailleerde informatie over bodemeigenschappen en bodem-heterogeniteit is noodzakelijk om de effecten van bodems op vegetatie te kunnen bestuderen. Deze informatie is met name belangrijk voor mijn studiegebied, omdat de heterogeniteit van de bodem kan toenemen door de aanwezigheid van ADE. Om die reden, heb ik uitgebreide bodempeilingen uitgevoerd (appendix, hoofdstuk 2 en 3), die me vervolgens in staat stelden om de relatie te bestuderen tussen precolumbiaanse bewoning en veranderingen in de concentratie van voedingstoffen in de bodem. Ik stelde vast dat bodems in het bos in de zuidelijke Amazone vruchtbaarder zijn dan hun tegenhangers in de centrale en oostelijke Amazone. Daarnaast heeft precolumbiaanse bewoning in het studiegebied lokaal geleid tot een verrijking van de bodem, middels een toename van de fosfor-, calcium- en kaliumconcentratie en door een verhoging van de pH. Met deze informatie kon ik specifieke hypotheses testen over de effecten van bodemvruchtbaarheid op de vegetatie.

Over het algemeen beïnvloedt de bodemvruchtbaarheid de samenstelling van kruiden (Angiospermae), varens en palmen in de ondergroei van het Amazonewoud. De toename van de bodemvruchtbaarheid op ADE zou dus ook een effect kunnen hebben op de samenstelling van deze kruiden, varens en palmsoorten. In hoofdstuk 2, heb ik de effecten onderzocht van ADE op de compositie, rijkdom en abundantie van soorten in de onderlaag van het bos (varens, kruiden en palmen). Allereest, heb ik uitgebreide bodempeilingen uitgevoerd die me vervolgens in staat stelde plekken in het bos aan te wijzen met een hogere beschikbaarheid van voedingsstoffen en met bewijs voor menselijke bewoning in het verleden (bv. de aanwezigheid van potscherven). Vervolgens, heb ik bodemvariabelen die geassocieerd worden met ADE, zoals Ca, P, en de pH van de bodem, gecorreleerd met de compositie, rijkdom en abundantie van plantensoorten. Ik vond dat de aanwezigheid van ADE een gradiënt creëert in bodemvoedingstoffen en pH, en zo de compositie van planten in de onderlaag van het bos beïnvloedt, met name die van varens en palmen. De hogere concentratie voedingsstoffen en meer neutralere pH van ADE bodems kwamen overeen met een afname in de rijkdom aan soorten varens. Mijn conclusie is dat de huidige compositie van de plantengemeenschap in de onderlaag van het bos in La Chonta een afspiegeling is van veranderingen van de bodem door precolumbiaanse bewoners.

Heterogeniteit in bodemvruchtbaarheid in het Amazonebekken is tot stand gekomen door variaties in de geologische geschiedenis en geomorfologische processen. Deze heterogeniteit veroorzaakt verschillen op grote schaal in de structuur en dynamiek van bossen in de Amazone. Op een kleinere schaal is het effect van bodem op de bosdynamiek echter nog slecht onderzocht. De studie van ADE biedt de mogelijkheid om te onderzoeken hoe een toename in bodemvruchtbaarheid de structuur en dynamiek van bossen beïnvloedt op een lokale schaal. In hoofdstuk 3, heb ik het effect van ADE op boseigenschappen geëvalueerd, zoals lichtbeschikbaarheid, het oppervlak van de stammen van de aanwezige bomen (grondvlak), de hoeveelheid lianen in het bos en het successiestadium. Het laatste werd bepaald aan de hand van de aanwezigheid van pioniers, langlevende pioniers, gedeeltelijk-schaduwtolerante en schaduwtolerante boomsoorten in het bos. Daarnaast heb ik onderzocht hoe ADE effect heeft op componenten van de bosdynamiek: boomgroei (in grondvlak), aanwas en sterfte van bomen. Verassend was dat ik slechts voor enkele boseigenschappen en componenten van de bosdynamiek een effect vond van de vruchtbare ADE. De pH van de bodem had een significante invloed op bosstructuur en bosdynamiek. Een hogere pH van de bodem leidde tot een hogere ingroei van middelgrote bomen (met een stamdiameter tussen de 20 en 40 cm) en tot een lagere sterfte van grote bomen (stamdiameter > 40 cm). Het belangrijkste effect van de bodem pH was echter op het grondvlak van de stammen van de aanwezige bomen en het successie stadium. Deze factoren hadden weer een direct effect op de groei van middelgrote bomen. Samenvattend heb ik gevonden dat in dit bos aan de zuidelijke rand van het Amazonebekken, andere factoren dan ADE (of de bodem in zijn algemeen) een sterkere invloed hebben op de bosdynamiek, zoals bijvoorbeeld de lichtbeschikbaarheid en hoeveelheid lianen in het bos.

Een hogere beschikbaarheid van voedingsstoffen in de bodem kan de groeisnelheid van planten vergroten en hun sterftekans verlagen. Planten reageren op de beschikbaarheid van voedingsstoffen door aanpassingen in diverse eigenschappen: biomassa-allocatiepatronen, morfologie, chemische samenstelling en fysiologie van weefsels. Deze aanpassingen maken een succesvolle vestiging en regeneratie van planten mogelijk. De hogere concentratie voedingstoffen in ADE in vergelijking tot natuurlijke bodems zou de groei van tropische bomen kunnen versterken. In hoofdstuk 4 heb ik de effecten van ADE bestudeerd op de groei, morfologie en fysiologie van zaailingen van 17 boomsoorten uit La Chonta in een kas experiment. Ik vond dat bodemtype een effect had op 10 van de 24 gemeten eigenschappen van de zaailingen. De zaailingen investeerden niet sterker in de productie van wortels (voor de opname van schaarse voedingstoffen) wanneer ze groeiden in niet-ADE dan in ADE, maar ze investeerden wel meer in bladeren en bladoppervlakte wanneer ze groeiden in ADE (om de opname van licht te vergroten). Dit leidde echter niet tot een verhoogde groei in ADE. De boomsoorten reageerden verschillend op de toename van de Ca concentratie in de bodem, die 2.4 keer zo hoog was in de ADE dan in de niet-ADE. Sommige soorten leken te lijden aan calciumvergiftiging, hetgeen vooral duidelijk werd door de verhoogde sterfte van zaailingen in ADE. Andere soorten lijden aan een disbalans in de verhouding van concentraties voedingsstoffen in de bladeren; weer andere soorten kregen een verhoogde Ca, P en N concentratie in hun bladeren. Alleen in deze laatste groep, de zogenaamde voedingsstof-accumulatoren, was er een positieve relatie tussen Ca concentratie in het blad en de groeisnelheid van zaailingen. In tegenstelling tot onze verwachting, zorgde ADE niet voor een verhoogde groeisnelheid van zaailingen. Het vermogen van planten om ADE te koloniseren hangt mogelijk af van hun reactie op hogere Ca concentratie in de bodem en van hun capaciteit om de calcium concentraties in hun weefsel te reguleren en zo hun voeding te balanceren.

In dit bos in het zuidelijke deel van het Amazonebekken is de toename van voedingstoffen in de bodem een nalatenschap van precolumbiaanse bewoning. De hierdoor ontstane toevoeging van voedingsstoffen veroorzaakt een verandering in de samenstelling van plantensoorten in de onderlaag van het bos, het vermindert de diversiteit van varensoorten, en heeft een bescheiden effect op de structuur en dynamiek van het bos. De toename van voedingsstoffen, en dan met name die van Ca, kan zowel een positief als negatief effect hebben en zo resulteren in lange-termijn effecten op de soortensamenstelling van het bos.

Resumen

El bosque Amazónico es usualmente visto como el arquetipo del bosque prístino, nunca transformado por los humanos; pero esta visión romántica está lejos de ser una realidad. En los últimos años, se ha incrementado la evidencia de prolongada y extensa modificación del paisaje por los humanos. El registro de procesos tales como la habitación permanente, expansión y contracción de poblaciones humanas no han sido siempre obvios in la Amazonía, dejando leves huellas en el paisaje que no son evidentes a simple vista. Un ejemplo de este, menos evidente, registro es la modificación del suelo conocida como Suelos Negros Amazónicos (ADE por sus siglas en inglés) o *terra preta* (tierra negra en Portugués), las cuales son el producto de la acumulación de residuos de la habitación humana permanente o semipermanente. Su nombre hace referencia al color negro de estos suelos, lo cual es una consecuencia de la acumulación de materia orgánica y piezas de carbón. Estos suelos contienen altos niveles de fósforo, calcio (originados principalmente de residuos de huesos), y nitrógeno que incrementa la fertilidad de suelos que, por lo general, son pobres en nutrientes, y por lo tanto, favorecen las actividades agrícolas en ellos. Los suelos negros Amazónicos (ADE) se distribuyen en Brasil, Bolivia, Colombia y Perú, y se estima que podrían ocupar hasta un 3% de la cuenca Amazónica.

Una de las consecuencias de la disminución en las poblaciones humanas tras el encuentro con los colonizadores provenientes de Europa, ha sido el abandono de sitios donde ADE ha sido formada, generando un nuevo crecimiento de la vegetación. Hasta la fecha, los efectos de ADE en el bosque que ha crecido después del abandono de las tierras por los indígenas, no han sido extensamente evaluados y solo actualmente comenzamos a entender las consecuencias de la habitación de los humanos en el pasado en la composición y estructura del bosque en la Amazonía. En la presente tesis, evalué los efectos de ADE en el bosque que ha crecido después del abandono por los indígenas en el sitio conocido como La Chonta, situado en el límite sur de la cuenca Amazónica en Bolivia. Inicialmente, evalué la magnitud de los cambios en el suelo como consecuencia de la ocupación humana. Para este fin, hice un mapa detallado de los suelos de 12 parcelas de cerca de 27ha cada una. Posteriormente, estudie como los cambios en el suelo afectan la composición de especies en el sotobosque (Capitulo 2), la estructura y la dinámica del bosque (Capitulo 3), y finalmente determine como las plántulas de las especies de árboles responden a los cambios en las propiedades del suelo que son causados por el hombre (Capitulo 4).

Información detallada de las características y la heterogeneidad del suelo in el paisaje es necesaria para evaluar los efectos del suelo en la vegetación. Esto fue incluso más importante en el sitio de mi investigación, ya que la heterogeneidad del suelo, se incrementa con la presencia de ADE. Por lo tanto, realice una descripción detallada (Apéndice, Capitulo 2, Capitulo 3) esto me ayudo a entender la relación entre la ocupación humana en la chonta y los cambios en la concentración de los nutrientes del suelo. Gracias a esta información, pude determinar que los suelos en la región sur de la Amazonía son más fértiles que en las regiones central y este. También encontré que la presencia de humanos en el pasado resulto en el enriquecimiento del suelo, especialmente por un incremento en la concentración de fosforo, calcio, potasio, y un incremento en el pH del suelo. Así, usando esta información, pude evaluar hipótesis especificas acerca de los efectos de la fertilidad del suelo en la vegetación que crece en estos suelos.

En el bosque Amazónico en general, las características del suelo tiene influencia en la composición de plantas herbáceas, helechos y palmas del sotobosque. Así, un incremento en la fertilidad del suelo en ADE se espera que afecte la distribución de las especies herbáceas, los helechos y las palmas del sotobosque. En el Capítulo 2, evalué el efecto de ADE en la composición, riqueza y abundancia de las especies del sotobosque (helechos, hierbas y palmas). Primero determine los sitios del bosque que contenían mayor cantidad de nutrientes y evidencia de ocupación humana en el pasado (por ejemplo, presencia de cerámica). Posteriormente, correlacione las variables del suelo que usualmente se asocian con ADE, tales como Ca, P, y pH del suelo con la composición de especies, la riqueza y la abundancia de individuos. Encontré que la presencia de ADE crea un gradiente de nutrientes y pH en el suelo, que cambia la composición de especies del sotobosque, especialmente de helechos y palmas. Además, una mayor cantidad de nutrientes en el suelo y un pH más neutral en ADE está relacionado con una disminución en la riqueza de especies de helechos. En conclusión, la composición actual del sotobosque en La Chonta puede ser considerada como un reflejo de la modificación del suelo realizada por los humanos en el pasado.

La heterogeneidad de los suelos en la Amazonía proviene de su historia geológica y de los procesos geomorfológicos de esta área. Esta heterogeneidad determina la estructura y la dinámica del bosque a través de toda la región. Sin embargo, a escala local, el papel que desempeñan los suelos en la dinámica del bosque aun no es completamente entendido. El estudio de ADE representa una oportunidad para evaluar como el incremento en la fertilidad del suelo puede afectar la estructura y la dinámica del bosque a escala local. En el Capítulo 3, evalué el efecto de ADE en los atributos del bosque, tal como el área basal del bosque, la infestación de lianas en los árboles, y la composición relacionada con la sucesión secundaria del bosque, que está definida por la presencia relativa de especies pioneras, pioneras de larga vida, especies parcialmente tolerantes a la sombra, y especies completamente tolerantes a la sombra. Adicionalmente, evalué el efecto de ADE en los componentes individuales de la dinámica del bosque: crecimiento del área basal, reclutamiento de árboles y mortalidad. Sorprendentemente, entontare que ADE afecto pocos atributos del bosque y pocos componentes de la dinámica del bosque. El pH del suelo fue una de las variables edáficas que significativamente explico la estructura y la dinámica del bosque. Un suelo con alto pH incremento el reclutamiento de árboles de tamaño intermedio (con diámetro del tallo entre 20 y 40 cm) y disminuyó la mortalidad de árboles grandes (con diámetro del tallo mayor a 40 cm). Sin embargo, el efecto más importante del pH del suelo fue en el área basal inicial en las parcelas evaluadas y la composición relacionada con la sucesión secundaria; lo cual, directamente afecto el crecimiento en el área basal de árboles de tamaño intermedio. En conclusión, con este estudio encontré que la dinámica del bosque está más fuertemente determinada por la disponibilidad de luz y la infestación por lianas de los árboles, que por ADE o por los suelos en general.

El incremento en los nutrientes del suelo puede causar respuestas en las plantas que a la vez promueven el incremento en tasas de crecimiento y disminución en las tasas de mortalidad. Las plantas responden a la disponibilidad de nutrientes en el suelo por medio de un conjunto de rasgos: ajustes en los patrones de redistribución de biomasa a las raíces o los tallos, ajustes morfológicos, ajustes en la fisiología en general y en la química de los tejidos, lo cual permite que las plantas tengan un establecimiento exitoso y se puedan regenerar en el bosque. Una mayor cantidad de nutrientes como la que se encuentra en las ADE, en comparación con los suelos naturales, podrían mejorar el crecimiento de las especies de árboles tropicales. En el Capítulo 4, estudie el efecto de ADE en el crecimiento, la morfología y la fisiología de plántulas en un experimento de invernadero en el cual cultive 17 especies de árboles tropicales del bosque de La Chonta en ADE y no-ADE. Como resultado de este experimento, encontré que el tipo de suelos provocó un cambio en 10 de 24 rasgos evaluados en las plántulas. Las plántulas no invirtieron más biomasa en las raíces en no-ADE (para tomar recursos más escasos del suelo) pero invirtieron más biomasa en hojas y área foliar en ADE (para incrementar captura de luz). Sin embargo, estos cambios morfológicos no conllevaron a un incremento en la tasa de crecimiento. De forma interesante, las especies respondieron diferente a un incremento en la concentración de calcio en el suelo, el cual fue 2.4 veces mayor en ADE que en non-ADE. Al parecer, algunas especies fueron afectadas por toxicidad de Ca como sugiere un incremento en la mortalidad de plántulas en ADE; otras especies mostraron un desbalance en los nutrientes lo cual fue observado por cambios en la estequiometria de los nutrientes en las hojas. Por ultimo otras especies incrementaron Ca, P y N concomitantemente en las hojas cuando crecieron en ADE. Solo este último grupo de especies, las cuales tienden a acumular nutrientes, mostró una relación positiva entre la concentración de Ca en las hojas y la tasa de crecimiento de las plántulas. En contradicción con las predicciones establecidas, ADE no determina un incremento en el crecimiento de las plántulas. La habilidad de las plantas para colonizar sectores de ADE en el bosque, podría depender de las respuestas de las especies al incremento en el Ca del suelo y su capacidad para regular el Ca en los tejidos que permita un equilibrado balance de otros nutrientes en la planta.

En resumen, en este bosque localizado en el sur de la Amazonía, el incremento en la concentración de nutrientes en el suelo es el legado de los humanos que lo han habitado desde tiempos pasados. La adición de nutrientes en el suelo causo cambios en la composición de las especies del sotobosque, y disminuyo la riqueza especies de helechos y tuvo un efecto menor en la estructura y la dinámica del bosque. Incrementos de los nutrientes en el suelo, específicamente en Ca, pueden causar respuestas positivas y negativas en las especies de árboles, resultando en efectos que podrían tener impacto a largo plazo en la composición de especies arbóreas en el bosque.

Second workshop Terra Preta Program, Manaus, Brazil (2012)

Acknowledgments

Gabriel García Marquez (the famous Colombian writer) wrote: "*los seres humanos no nacen para siempre el día que sus madres los alumbran, sino que la vida los obliga a parirse a sí mismos una y otra vez*" (Humans are not born forever the day that their mothers deliver them; life makes them labor themselves time after time). These words perfectly describe what I have felt during my PhD. I have gained so much during this 4.5 years that it feels like I am born again both in science and personally, and in this process many people have contributed significantly.

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Short Biography

Estela Maria Quintero Vallejo was born in Medellin, Colombia on May 2nd,1975. She studied elementary and high school at Colegio San Juan Bosco. She studied Biology at Universidad de Antioquia, Medellin where she was active in student organizations, such as the National Association of Biological Science Students (ANECB, in Spanish).

During her studies, she did an internship at the Corporations for Biological Research (CIB, in Spanish) where she carried out her Bachelor's thesis on Mutagenesis of Bacteria to be used as safe biological control. In 2000, she participated in the Tropical Ecology and Conservation Course organized by Organization for Tropical Studies (OTS) in Costa Rica.

After graduating in 2000, she joined a young research program funded by Wildlife Conservation Society (WCS) in Cali, Colombia under the supervision of Carolina Murcia and Gustavo Kattan. In this three year program she carried out research on the restoration ecology of watersheds in the Andean cloud forests. She travelled to the USA in 2004 for her MSc studies at Department of Ecology, Evolution and Organismal Biology of Ohio State University. For her MSc thesis she worked on the life history and physiological strategies of native and invasive species of the prairies. In 2008 she returned to Medellin, where she was appointed as part time associate professor at the University of Antioquia, regional Campus. She taught courses on general ecology, evolution and plant physiology for 2 years. In 2011 she started her PhD in the *Terra Preta* interdisciplinary program at Wageningen University together with six other PhD students from Colombia and Brazil. This dissertation forms the result of her research work.

PE&RC Training and Education Statement

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

Review of literature (4.5 ECTS)

- The effects of anthropogenic dark earth on the composition, structure and dynamics of an Amazonian forest

Writing of project proposal (4.5 ECTS)

- The effects of anthropogenic dark earth on the composition, structure and dynamics of an Amazonian forest (2011)

Post-graduate courses (5.7 ECTS)

- Linear models; PE&RC (2011)
- Generalized models; PE&RC (2011)
- Linear mixed models; PE&RC (2011)
- Bayesian statistics; PE&RC (2011)
- Introduction to R; PE&RC (2012)
- Multivariate statistics; PE&RC (2012)
- Soil taxonomy; ISRIC (2014)

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

- Biotropica: shrub cover influence on seedling growth and survival following logging of a tropical forest
- Biotropica: how tightly linked are *pericopsis elata* (Fabaceae) patches to anthropogenic disturbances in South-eastern Cameroon? Some elements of a long story

Deficiency, refresh, brush-up courses (2 ECTS)

- Advanced course on forest ecology and forest management

Competence strengthening / skills courses (2.4 ECTS)

- Competence assessment; Wageningen Graduate School (2011)
- Information literacy and Endnote course; Wageningen Library (2011)
- Scientific writing; Wageningen in’to Languages (2014)

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

- PE&RC PhD Weekend (2011)
- PE&RC Day (2012)
- PE&RC Weekend (2014)
- PE&RC Day (2014)

Discussion groups / local seminars / other scientific meetings (6.95 ECTS)

- Terra Preta discussion group (2011)
- Terra Preta workshop; Wageningen, the Netherlands (2011)
- Wageningen evolution and ecology seminars (2011)
- Terra Preta workshop; Manaos, Brasil (2012)
- Ecological theory and application (2012)
- Terra Preta workshop; Leticia, Colombia (2013)

International symposia, workshops and conferences (3.8 ECTS)

- Annual meeting of Association for Tropical Biology; Bonito, Brasil (2012)
- Netherlands Annual Ecology Meeting (NAEM); Lunteren, the Netherlands (2014)

Supervision of a MSc student

- The effect of Amazonian dark earths on composition, diversity and density of understory herbs, ferns and palms in a Bolivian tropical forest