Pre-Columbian Nutrient Inputs into *Terras Pretas* and Raised Fields in Amazonia

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Abbreviations

AD	Anno Domini
ADE	Amazonian Dark Earth, Anthropogenic Dark Earth
ANCOVA	Covariance analyses
$\mathrm{Ba}_{\text{pseudo-tot.}}$	Pseudo-total barium
BC	In Chapter 2: biochar, in all other Chapters: before Christ,
BPCA	Benzenepolycarboxylic acids
BSTFA	Bis(trimethylsilyl)trifluoroacetamide
$\mathrm{Ca}_{\text{avail.}}$	Plant-available calcium
$\mathrm{Ca}_{\text{pseudo-tot.}}$	Pseudo-total calcium
CEC	Cation exchange capacity
$\mathrm{Cu}_{\text{pseudo-tot.}}$	Pseudo-total copper
DCA	3α,12α-Dihydroxy-5β-cholanoic acid
DCM	Dichloromethane
DNA	Deoxyribonucleic acid
DRIFT	Diffuse-reflectance infrared Fourier transformation
ECEC	Effective cation exchange capacity
EDS	Energy-dispersive X-ray spectroscopy
EPR	Electron paramagnetic resonance
GC	Gas chromatography
HDCA	3α,6α-Dihydroxy-5β-cholanoic acid
HMDS	Hexamethyldisilazane
ICP-OES	Inductively coupled plasma optical emission spectrometer
IDCA	7α,12α-Dihydroxy-5β-cholanoic acid
IS1	First internal standards
IS2	Second internal standard
$\mathrm{K}_{\text{avail.}}$	Plant-available potassium
$\mathrm{K}_{\text{pseudo-tot.}}$	Pseudo-total potassium
LCA	3α-Hydroxy-5β-cholanoic acid
LOD	Limit of detection
LOQ	Limit of quantification
$\mathrm{Mg}_{\text{avail.}}$	Plant-available magnesium
$\mathrm{Mg}_{\text{pseudo-tot.}}$	Pseudo-total magnesium
MS	Mass spectrometry

$N_{tot.}$	Total nitrogen
$Na_{avail.}$	Plant-available sodium
OC	Organic carbon
$P_{avail.}$	Plant-available phosphorus
$P_{pseudo-tot.}$	Pseudo-total phosphorus
RSD	Relative standard deviation
RT	Retention times
SEM	Scanning electron microscopy
SIM	Selected ion monitoring
SOM	Soil organic matter
SPE	Solid phase extraction
TLE	Total lipid extract
TMCS	Trimethylchlorosilane
TOC	Total organic carbon
TSIM	trimethylsilylimidazole
UDCA	3α,7β-Dihydroxy-5β-cholanoic acid
$Zn_{pseudo-tot.}$	Pseudo-total zinc

Summary

During the last decades, increasing evidence has shown impacts of pre-Columbian societies on Amazonian ecosystems. The extent to which Amazonian ecosystems, prior considered as ‘pristine’, are the legacy of pre-Columbian land use is currently highly debated. The idea of intense pre-Columbian human occupation in Amazonia has inspired optimism that the Amazonian ecosystems could be more resilient to intense human occupation than often feared. It is hoped that research on modification of ecosystems by pre-Columbian people could provide knowledge to develop sustainable agriculture in the humid tropics and beyond. In this context *terras pretas (de Índio)* and raised fields have received broad interest. T*erras pretas* are Anthrosols of pre-Columbian origin that are characterized by high contents of charred organic matter (black carbon, biochar) and high nutrient levels. The existence of *terras pretas* shows that the fertility of highly weathered tropical soils can be sustainably raised and long-term carbon sequestration is possible in these soils. Raised-field agriculture is a technique of plant cultivation in wetlands on artificial raised soil surfaces. Recently, pre-Columbian raised fields in French Guiana have aroused interest because they exhibit characteristics of self-organizing patchiness. Today, they provide habitats for plants, termites, ants and earthworms, which appear to stabilize the raised structures against erosion. In maintaining suitable habitats for themselves and other organisms, they preserve the raised structures.

Terras pretas as well as the remains of raised fields are frequently associated with highly weathered nutrient-poor soils. Nutrient incorporation thus must have been an important factor for the genesis of *terras pretas* and for a productive raised-field agriculture. However, little is known about nutrient sources in *terras pretas* and nutrient concentrations and sources in pre-Columbian raised fields. Knowledge about the deposition of nutrient-rich material through pre-Columbian land use is crucial for understanding intensity of pre-Columbian land use and population densities in Amazonia. In addition, such knowledge is necessary to develop sustainable agriculture in the humid tropics based on pre-Columbian land-use techniques.

The objective of this thesis is to investigate anthropogenic nutrient inputs into *terras pretas* and raised fields in Amazonia. Therefore existing knowledge about nutrient inputs into *terras pretas* in Amazonia and raised fields in Meso- and South America was discussed in two reviews, a biomarker method for the analysis of faeces inputs into soils was developed and *terras pretas* and raised fields were analysed.

The review about *terras pretas* showed that the deposition of several materials contributed to the high nutrient stocks in *terras pretas* and that the deposition of bones was an important source of nutrient in these soils. The hypothesis that faeces deposition contributed to the high nutrient stocks in *terras pretas* was investigated in this thesis by steroid analyses. Specific steroids, 5β-stanols, have a high concentration in faeces of humans and many animals. The relation of their amounts to the amounts of their precursors, Δ^5-sterols, and/or their 5α-isomers (the main reduction products of Δ^5-sterols in the environment) can be used to investigate the deposition of faeces. Steroid concentrations were analysed in samples from five *terras pretas* and adjacent reference soils. The concentration patterns of sterols in these soils showed that faeces of omnivores were deposited on *terras pretas*.

Although even small concentrations of 5β-stanols, if related to the concentrations of their precursors and 5α-isomers, allow reliable detection of enhanced faeces deposition, they provide only raw information about the origin of the faecal material. Analysis of bile acids can deliver more detailed information about the source of faeces than the analysis of stanols. Therefore, a method was developed enabling the quantification of 5β-stanols and their 5α-isomers together with Δ^5-sterols and bile acids based on a single lipid extraction. 5β-Stanones are assumed to have a potential as biomarkers comparable with 5β-stanols and their quantification was included in the method. The method was evaluated by standard addition to soil samples with a wide range of different physico-chemical properties including a *terra preta* and other Anthrosols. The standard contained 5β-stanols, 5 β -stanones and their 5α-isomers together with Δ^5-sterols and bile acids (19 substances). This evaluation showed that the method allows a precise quantification of stanols, stanones, Δ^5-sterols and bile acids with mean recoveries of individual substances ≥85%. The recoveries of biomarkers within each biomarker group did not differ significantly. Thus, the method is suitable for quantification of steroid patterns in soil samples. Data from the *terra preta* showed that the bile acid pattern in *terra preta* was typical for faeces of humans.

In this thesis, pre-Columbian raised-field sites in seasonally flooded savannas in French Guiana were studied in order to assess nutrient management in raised-field agriculture by pre-Columbian farmers and its biogeochemical legacy. At five raised-field sites, amounts of total organic carbon, total nitrogen and plant-available nutrients were analysed. Additionally, at two sites, pseudo-total element concentrations (extracted with aqua-regia) were analysed. Amount of total organic carbon was elevated in some raised fields in comparison to flat areas. The concentrations of pseudo-total phosphorus and total nitrogen were correlated with the amount of total organic carbon. Slightly elevated concentration of plant-available phosphorus was found in some raised fields in comparison to flat areas, but the concentration of plant-available phosphorus remained overall low. The enrichments observed could have been caused by the construction and maintenance of raised fields by pre-Columbian farmers using topsoil or plant material applied to soils, and by the effects of soil invertebrates. In the review of raised-field agriculture, it was concluded that fallow periods may have been necessary in pre-Columbian raised-field agriculture on nutrient-poor soils. Today, the savannas are fire-prone environments. Probably a reduction of the frequency and intensity of fires could have accelerated biomass build-up during fallow periods. Furthermore, biomass that grew in the wet basins between raised fields during fallow periods could have provided green manure for the raised fields.

No clear evidence was found showing that pre-Columbian raised-fields in Amazonia were fertilized with material from external sources and the review about *terras pretas* showed that they are unlikely the legacy of pre-Columbian agricultural fields. Thus, data in this thesis did not provide clear evidence for intentional fertilization of agricultural fields by pre-Columbian farmers with nutrient-rich material from external sources. Farming of raised fields on nutrient-poor soils probably required long fallow periods, supporting population densities that were lower than in most current estimates. However, faeces should be considered as nutrient source in estimations using nutrient amounts in *terras pretas* to calculate population densities in pre-Columbian Amazonia and they could be an important fertilizer for a sustainable agriculture in the humid tropics which imitates properties of *terras pretas*.

Zusammenfassung

Lange Zeit wurde angenommen, dass Amazonien in präkolumbischer Zeit kaum vom Menschen beeinflusst wurde. In den letzten Jahrzehnten mehrten sich jedoch die Hinweise auf eine erhebliche Umgestaltung der Ökosysteme Amazoniens durch die präkolumbische Bevölkerung. Zwar ist das Ausmaß der menschlichen Einflussnahme in dieser Zeit umstritten, die Idee eines relativ dicht besiedelten präkolumbischen Amazoniens weckte aber die Hoffnung, dass die dortigen Ökosysteme intensiver nutzbar sind, als oft angenommen wurde. Man erhofft sich daher, durch die Untersuchung der präkolumbischen Landnutzungsformen Erkenntnisse zu gewinnen, die zu einer Entwicklung nachhaltiger landwirtschaftlicher Landnutzungsformen in den humiden Tropen beitragen können. Die vorliegende Arbeit beschäftigt sich in diesem Zusammenhang mit *Terras Pretas* und präkolumbischen Hochbeeten (*raised fields*). *Terras Pretas* sind fruchtbare Anthrosole mit hohen Gehalten an Biokohle (*black carbon*) und Nährstoffen. Ihre Existenz zeigt, dass es möglich ist, die Fruchtbarkeit stark verwitterter tropischer Böden nachhaltig zu erhöhen und zugleich Kohlenstoff in diesen Böden zu speichern. Hochbeetlandwirtschaft wurde in Amazonien in saisonal überfluteten Gebieten betrieben. Hierbei wurden Kulturpflanzen auf künstlich erhöhten Oberflächen angebaut. Gebiete, die sich unter präkolumbischer Hochbeetlandwirtschaft befanden, weisen heute Parallelen zu natürlichen selbstorganisiert-strukturierten Ökosystemen (*self-organizing patchiness*) auf: Die erhöhten Strukturen der ehemaligen Hochbeete stellen ein Habitat für Pflanzen, Termiten, Ameisen und Regenwürmer dar. Diese Organismen erhalten die erhöhten Strukturen und damit ihre eigenen Habitate.

Terras Pretas und präkolumbische Hochbeete entstanden oft auf stark verwitterten, nährstoffarmen Böden. Eine Nährstoffzufuhr muss daher für die Genese der *Terras Pretas* und für eine produktive Hochbeetlandwirtschaft von großer Bedeutung gewesen sein. Über die Nährstoffquellen in *Terras Pretas* und über die Nährstoffgehalte und -quellen in präkolumbischen Hochbeeten war bisher nur wenig bekannt. Das Wissen darüber ist jedoch sowohl für die Rekonstruktion der präkolumbischen Landnutzung und Besiedlungsdichte, als auch für die gegenwärtige Entwicklung einer nachhaltigen Landwirtschaft von großer Bedeutung.

Ziel dieser Arbeit ist eine Untersuchung der Nährstoffeinträge in *Terras Pretas* und in präkolumbische Hochbeete. Die bisherigen Kenntnisse über Nährstoffeinträge in *Terras Pretas* in Amazonien und präkolumbische Hochbeete in Mittel- und Südamerika wurden in je einem Review diskutiert, eine Biomarkermethode zur Analyse von

Fäkalieneinträgen in Böden entwickelt sowie *Terras Pretas* und Hochbeetflächen untersucht. Der Review über *Terras Pretas* zeigte, dass die hohen Nährstoffgehalte der *Terras Pretas* auf den Eintrag verschiedener Materialien zurückgeführt werden können und dass v. a. der Eintrag von Knochen eine bedeutende Nähstoffquelle war. Die Hypothese, dass Fäkalieneinträge zu den hohen Nährstoffgehalten in *Terras Pretas* beitrugen, wurde in dieser Arbeit mithilfe von Steroidanalysen untersucht. In menschlichen Fäkalien und in den Fäkalien einiger weiterer Säugetiere sind charakteristische Steroide, die 5β-Stanole, in hoher Konzentration zu finden. Die Verhältnisse ihrer Gehalte zu den Gehalten ihrer Vorläufersubstanzen (Δ^5-Sterole) und ihrer 5α-Isomere (die häufigsten Reduktionsprodukte der Δ^5-Sterole in der Umwelt) können für die Untersuchung von Fäkalieneinträgen genutzt werden. In fünf *Terras Pretas* und angrenzenden Referenzböden wurden die Steroidegehalte analysiert. Diese Studie zeigte, dass das Muster der Steroidkonzentrationen in *Terras Pretas* für den Eintrag von Fäkalien von Omnivoren charakteristisch ist.

Die Analyse der Gehalte an 5β-Stanolen in Beziehung zu den Gehalten ihrer 5α-Isomeren und ihrer Vorläufersubstanzen ermöglicht zwar eine sensitive Untersuchung von Fäkalieneinträgen, liefert aber keine genauen Informationen über die Art der Fäkalien. Dagegen ermöglicht eine Untersuchung der Gallensäuremuster in Böden eine genauere Bestimmung der eingetragenen Fäkalien. In dieser Arbeit wurde daher eine Methode zur gleichzeitigen Quantifizierung von 5β-Stanolen und ihrer 5α-Isomere zusammen mit Δ^5-Sterolen und Gallensäuren in Lipidextrakten von Bodenproben entwickelt. Da 5β-Stanone ein ähnliches Potenzial als Biomarker haben wie 5β-Stanole, wurden sie in die Methodenentwicklung einbezogen. Die Methode wurde durch Standardaddition zu Böden mit stark variierenden Eigenschaften evaluiert. Eine *Terra Preta* und weitere Anthrosole waren in der Auswahl der Böden für die Methodenevaluierung enthalten. Der eingesetzte Standard enthielt 5β-Stanole, 5β-Stanone und ihre 5α-Isomere, Δ^5-Sterole und Gallensäuren. Die Evaluierung zeigte, dass die Methode eine präzise Quantifizierung der Biomarker mit einer mittleren Wiederfindung ≥85% je Substanz ermöglicht. Die Wiederfindungen der einzelnen Biomarker unterschieden sich nicht signifikant, sodass die Methode für eine Untersuchung der Biomarkermuster geeignet ist. Das Gallensäuremuster der zur Methodenevaluierung herangezogenen *Terra Preta* war charakteristisch für menschliche Fäkalien.

Um das Nährstoffmanagement in der präkolumbischen Hochbeetlandwirtschaft und seine biogeochemischen Auswirkungen auf die heutigen Nährstoffgehalte in den

ehemaligen Hochbeeten zu untersuchen, wurden in dieser Arbeit präkolumbische Hochbeete in den saisonal überfluteten Savannen Französisch Guayanas untersucht. An fünf Standorten wurden die Gehalte an organischem Kohlenstoff, Gesamtstickstoff und pflanzenverfügbaren Nährstoffen analysiert. Zusätzlich wurden an zwei Standorten pseudototale Elementkonzentrationen gemessen (mit Königswasser extrahiert). In einigen Hochbeeten wurden – im Vergleich zu ebenen Savannenflächen – erhöhte Gehalte an organischem Kohlenstoff gefunden. Die pseudototalen Phosphorkonzentrationen und die Gesamtstickstoffgehalte korrelierten mit den Gehalten an organischem Kohlenstoff. Eine geringfügige Erhöhung der pflanzenverfügbaren Phosphorkonzentrationen wurde an einigen Standorten gefunden, jedoch waren die Gehalte an pflanzenverfügbarem Phosphor niedrig. Die Erhöhung der organischen Kohlenstoffkonzentrationen kann durch das Aufbringen von Oberboden und Pflanzenmaterial beim Bau und der Pflege der Hochbeete verursacht worden sein. Eine Erhöhung sowohl der organischen Kohlenstoffkonzentration, als auch der Gehalt an pflanzenverfügbarem Phosphor in den Hochbeeten kann jedoch auch durch die Tätigkeit der Organismen, die heute die Hochbeete besiedeln, erklärt werden. In dem Review über Hochbeetlandwirtschaft wird die Schlussfolgerung gezogen, dass möglicherweise Bracheperioden von entscheidender Bedeutung für die Fruchtbarkeit der präkolumbischen Hochbeete auf nährstoffarmen Böden waren. Heute sind die Savannen durch häufige Brände geprägt. Gegebenenfalls wurde die Biomasseproduktion während der Brachezeiten durch eine Verminderung der Brände beschleunigt. Die Vegetation, die während der Brachezeiten in den Senken zwischen den Hochbeeten wuchs, könnte zur Gründüngung genutzt worden sein.

Es wurden keine deutlichen Hinweise auf eine Ausbringung von Dünger aus einer externen Quelle auf die präkolumbischen Hochbeete in Amazonien gefunden. Der Review über *Terras Pretas* zeigte, dass *Terras Pretas* wahrscheinlich nicht das Produkt gezielter Düngung landwirtschaftlicher Felder sind. In dieser Arbeit wurden also keine Hinweise auf eine gezielte Düngung landwirtschaftlicher Felder mit von außen eingebrachtem Material zu präkolumbischer Zeit in Amazonien gefunden. Eine Hochbeetlandwirtschaft auf Flächen mit nährstoffarmen Böden benötigte wahrscheinlich lange Brachezeiten und konnte daher vermutlich nur eine geringere Bevölkerung ernähren, als bisher angenommen. Fäkalieneinträge sollten jedoch berücksichtigt werden, wenn anhand von Nährstoffkonzentrationen in *Terras Pretas* auf die Besiedlungsdichte Amazoniens geschlossen wird. Sie könnten als Dünger für die Entwicklung einer Landwirtschaft, die die Bodeneigenschaften der *Terras Pretas* nachbildet, von Bedeutung sein.

Chapter 1

General introduction and objectives

1.1 General introduction

1.1.1 Recent problems of land use in Amazonia

Amazonian ecosystems exhibit a high biodiversity and are of high importance for the regulation of the Earth's climate (Bonan, 2008; Junk and Piedade, 2004; Lewis, 2006; Perfecto and Vandermeer, 2008; Trumper et al., 2009). During the last decades, ecosystems have been destroyed rapidly (Davidson et al., 2012; Fearnside, 2005; Junk and Piedade, 2004; Lewis, 2006; Trumper et al., 2009). Increasing population pressure caused expansion of the area under cultivation as well as overexploitation of agricultural areas (DeFries and Rosenzweig, 2010; Fearnside, 2005; IPCC, 2000). Boosted by the Green Revolution, agricultural intensification increased productivity but at a high environmental cost (Perfecto and Vandermeer, 2008). Further agricultural exploitation, especially large-scale cattle raising, contributed to the loss of Amazonian ecosystem (Fearnside, 2005; Junk and Piedade, 2004).

In Amazonia, highly weathered soils dominate in areas that are not periodically flooded; the so-called *terra firme* (Quesada et al., 2011; Sombroek, 1966, 2000). Following logging, these soils can be used for agriculture only for short periods of time. After a few years considerable additions of mineral fertilizers are necessary to maintain plant production (Tiessen et al., 1994). Therefore, extensive slash-and-burn agriculture with long fallow periods between brief farming periods has been considered to be the most adopted agriculture in these areas (Fearnside, 1985; Kleinman et al., 1995). More fertile soils can be found e.g. in floodplains of rivers that carry sediments from the Andean cordillera (*várzea)* (Fearnside, 1985; Junk and Furch, 1993; Prance, 1979; Sombroek, 2000). However, variability of the extent of seasonally flooding limits agriculture in these areas (Fearnside, 1985). Thus, for many years, it has been assumed that environmental limitations inhibit intensive agriculture in Amazonia (Meggers, 1954, 2001).

1.1.2 Pre-Columbian anthropogenic ecosystem modifications as model for sustainable agriculture in the humid tropics

Due to environmental constraints on food production, it was assumed that pre-Columbian population densities in Amazonia were low (Meggers, 1954, 2001). However, during the last decades, evidence has increased showing impacts of pre-Columbian societies on Amazonian ecosystems (Denevan, 2001; Erickson, 2008; Heckenberger and

Neves, 2009; Iriarte, 2007; Petersen et al., 2001). The extent to which Amazonian ecosystems, prior considered as ‘pristine’, are the legacy of pre-Columbian land use is currently highly debated (Barlow et al., 2012; Clement and Junqueira, 2010; Levis et al., 2012; McMichael et al., 2012). The idea of intense pre-Columbian human occupation in Amazonia has inspired optimism that the Amazonian ecosystems could be more resilient to intense human occupation than often feared (McKey et al., 2010). It is hoped that research on modification of ecosystems by pre-Columbian peoples could provide knowledge to develop sustainable agriculture in the humid tropics (Glaser, 2007; Heckenberger et al., 2008; McKey et al., 2010; Sombroek et al., 2003; Woods and McCann, 1999). In this context, Amazonian Dark Earths and raised fields have received broad interest, because they are assumed to be the legacy of once widely practiced, but now virtually extinct, land-use techniques in Amazonia that have a real potential for contributing to the design of sustainable agroecosystems today (Glaser, 2007; Lehmann et al., 2006; McKey et al., 2010; Sombroek et al., 2002; Woods and Denevan, 2009).

Amazonian Dark Earths or *terras pretas (de índio)* are patches of dark coloured, highly fertile, Anthrosols of pre-Columbian origin (Glaser and Woods, 2004; Lehmann et al., 2003b; Petersen et al., 2001; Sombroek, 1966; Woods et al., 2009). The main biogeochemical features which distinguish these soils from surrounding soils are a constantly high cation exchange capacity and high nutrient levels (Costa and Kern, 1999; Falcão et al., 2009; Glaser et al., 2001b; Lehmann et al., 2003a; Sombroek, 1966). The high cation exchange capacity is maintained by a stable pool of soil organic matter (Glaser, 2007; Glaser et al., 2001a, 2001b; Zech et al., 1990). Glaser et al. (2001a) identified charred organic matter (black carbon; biochar) as a key factor for the stability of the organic matter in these soils. Oxidation of black carbon produced carboxylic groups on the edges of the aromatic backbone, which increased the cation exchange capacity (Glaser et al., 2000). Due to enhancement of cation exchange capacity, carbon storage and further effects, the application of charred organic matter on soils is considered to have a high potential for the development of agricultural techniques that could be sustainable and could counteract anthropogenic climate change on the *terra firme* in Amazonia and areas beyond (Glaser, 2007; Glaser et al., 2002; Lehmann et al., 2006; Steiner et al., 2004).

It is still not clear if *terras pretas* are the legacy of intentional improvement of soils by pre-Columbian farmers or if they were created by change through settlement activities (Glaser, 2007). Some authors differentiate among types of dark-coloured Anthrosols in Amazonia (e.g. *terra mulata* versus *terra preta*) (McCann et al., 2001; Neves et al., 2003;

Sombroek, 1966; Woods and McCann, 1999). According to McCann et al. (2001), *terras mulatas*, like *terras pretas*, have elevated amount of soil organic matter compared with surrounding soils, but the nutrient concentrations are not, or only slightly, enriched compared to the surrounding soils. It is speculated that these soils are the product of mulching and burning of agricultural fields by pre-Columbian farmers (McCann et al., 2001; Neves et al., 2003; Woods and McCann, 1999). However, only limited data from *terras mulatas* exist and it is questioned if they can be distinctly differentiated from *terras pretas* or represent a gradual transition between *terras pretas* and surrounding soils (Fraser et al., 2011).

More clearly than *terras pretas* and *terras mulatas*, the remains of pre-Columbian raised fields are the legacy of pre-Columbian agricultural fields. Raised-field agriculture is an agricultural technique of plant cultivation in wetlands on artificial raised soil surfaces. Vestiges of ancient raised-field agriculture cover vast areas of seasonally flooded savannas in the periphery of Amazonia. They have been found in the Llanos de Mojos of Bolivia (Denevan, 1966; Erickson, 1995; Saavedra, 2009; Walker, 2004), in the Llanos of Venezuela (Denevan and Zucchi, 1978; Spencer, 2000; Zucchi and Denevan, 1972) and in the coastal savannas of the Guianas (Rostain, 2008a, 2010; Versteeg, 2008).

Centuries after being abandoned, the raised structures in Amazonia persist, despite being subjected to highly erosive tropical rainfall and to fires that seasonally reduce vegetation. McKey et al. (2010) and Renard et al. (2013) analysed small raised fields (ca. 1.2 m in diameter and ca. 0.18 m high) in the coastal savannas of French Guiana. They provided evidence that re-engineering by non-human organisms has enabled them to persist. According to this idea, raised-field farmers acted as ecosystem engineers by creating raised fields. These patches of soil remain above the water level during seasonal flooding, providing habitats for soil ecosystem engineers, mainly termites, ants, earthworms and plants. By transporting material to the raised fields and by stabilizing them against erosion, these ecosystem engineers maintain the raised structures. Thus, vestiges of raised fields in French Guiana may be examples of coupled systems of human and natural engineering. The physically structured raised-field areas are described by McKey et al. (2010) and Renard et al. (2013) as alternative stable states of marshy savannas. The savannas would have remained in a stable state (relatively flat and homogeneous) had raised fields not been constructed. After being abandoned, the raised fields would have eroded and the savannas would have returned to the flat state if the raised structures were not maintained by the ecosystem engineers. McKey et al. (2010) suggested that pre-Columbian raised-field agriculture in coastal savannas of French

Guiana could serve as a model for how self-organizing processes in coupled human and natural systems could be exploited in agriculture.

1.1.3 Nutrients and nutrient sources in pre-Columbian modified soils

While little is known about the nutrient contents in raised fields, it is well described that *terras pretas* typically contain high nutrient concentrations. First of all, they are enriched in plant-available P (Falcão et al., 2009; Glaser, 2007; Lehmann et al., 2003a; Smith, 1980; Sombroek, 1966) Also total P is several times higher in *terras pretas* than in surrounding soils (Costa and Kern, 1999; Lehmann et al., 2003a). Plant-available and total Ca, N and Mg are strongly enriched in relation to surrounding soils and amounts of K, Zn and Mn were also found at higher levels in *terras pretas* (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Lehmann et al., 2003a; Sombroek, 1966).

Terras pretas can be related to various soil types, but most commonly developed from Ferralsols and Acrisols (Kern et al., 2003). The remains of raised fields can also be found on highly weathered nutrient-poor soils (Boulet, 1975; Leprun et al., 2001; Lombardo et al., 2011; Rostain, 2008a, 2010). Nutrient incorporation thus must have been an important factor for the genesis *terras pretas* and for a productive raised-field agriculture.

Fertilization of agricultural areas as well as concentration of nutrients in settlement areas through the deposition of debris can cause an elevation of nutrient and carbon concentrations in soils that can persist for long periods of time (Linderholm and Lundberg, 1994; McLauchlan, 2006; Oonk et al., 2009; Woods, 2003a). In Meso- and South America, fertilization with material from external sources seemingly was a component of pre-Columbian agriculture. Reviewing the evidence, Denevan (2001) considered that fertilization with faeces was probably of major importance in Andean agriculture. In the Valley of Mexico compost, probably including faeces, was used for fertilization (Armillas, 1971). On the Pacific coast of South America, several reports exist of fish being used as fertilizer in addition to guano (Denevan, 2001). However, little is known about how widespread and important soil fertilization with material from external sources was in pre-Columbian agriculture in Amazonia.

Only a few studies of the nutrient sources for *terras pretas* have been carried out so far (Glaser, 2007) and it is not known if raised fields in Amazonia were fertilized in the past.

1.1.4 Methods for identification and quantification of nutrient inputs into soils

Classically, phosphate was analysed in soils to detect ancient human activities areas (Bethel and Máté, 1989; Eidt, 1977; Holliday and Gartner, 2007; Woods, 2003a). Phosphorus is suitable for the analysis of human material deposition because of its universal association with human activities (Eidt, 1977). Elevated phosphorus concentrations can originate from various different cultural debris (Holliday and Gartner, 2007; Woods, 2003a). Phosphate can be strongly bound in many soils, which makes it useful to analyse deposition of nutrient-rich materials in the past (Eidt, 1977; Holliday and Gartner, 2007).

Due to instrumental developments in the last decades, simultaneous analyses of several elements in soil extracts was facilitated. In this way, multi-element analyses were used in an increasing number of studies to analyse anthropogenic inputs (Linderholm and Lundberg, 1994; Middleton, 2004; Oonk et al., 2009; Wilson et al., 2008). Analyses of several elements in addition to phosphorus can deliver hints about the kind of the material which was deposited. In this way, various element ratios can be used to analyse the nutrient sources, e.g. bones contain high amounts of P and Ca (Lima et al., 2002; Wilson et al., 2008). Ash contains high amounts of Ca, K and Mg (Woods, 2003a). Cu and Zn can be found in relatively high concentrations in combination with high P concentration in excrements (Woods, 2003a). High amounts of Ba and Ca can deliver hints for the deposition of shells (Puchelt, 1978). Various further element concentrations can be characteristic for different functional areas in human activity areas and for different materials deposited trough human actions (Hjulström and Isaksson, 2009; Middleton, 2004; Oonk et al., 2009; Parnell and Terry, 2002; Wilson et al., 2008).

Whereas the classical analyses of soil phosphorus content in archaeological context used extraction procedures developed for the analyses of plant-available phosphorus, different extraction procedures were employed meanwhile including total and pseudo-total extractions (Holliday and Gartner, 2007; Linderholm and Lundberg, 1994; Wilson et al., 2006). Pseudo-total extraction do not completely extract all elements, e.g. tectosilicates are not completely dissolved (Blume et al., 2000; Schwartz and Kölbel, 1992). However, they result in complete extraction of anthropogenically deposited elements in most cases (Blume et al., 2000; Linderholm and Lundberg, 1994; Wilson et al., 2006). It is a matter of debate if easily extractable element concentrations or total and pseudo-total element concentrations are more suitable to detect human soil modification. On the one hand, human-induced changes in the concentrations of easily extractable fractions has been criticized to persist in soil less time compared to changes in the pseudo-total and total concentrations. On the other hand, differences in easily extractable concentration caused by human soil modifications could be overwhelmed by much higher natural background concentrations obtained by pseudo-total and total extractions (Holliday and Gartner, 2007; Middleton, 2004; Middleton and Price, 1996; Wilson et al., 2006). More detailed information on nutrients in anthropogenic modified soils can be obtained by sequential extractions (Eidt, 1977; Oonk et al., 2009; Wilson et al., 2006). However, these kinds of extractions are more time-consuming making them less practical for mapping studies and comparable studies that depend on the analyses of a great number of samples.

An alternative approach to analyses of nutrient concentrations and fractions in soil to obtain information about their sources is the analyses of stable isotope ratios, specific biomolecules and their combination. Different materials deposited on soils in the past can cause differences in the bulk soil $\delta^{13}C$ and $\delta^{15}N$ values. Data from long-term experiments showed that fertilization with animal manure causes higher bulk $\delta^{15}N$ values in soils (Bol et al., 2005; Singh Antil et al., 2005). Koerner et al. (1999) showed that animal manure application on agricultural areas is still detectable in $\delta^{15}N$ values in soils in France about seventy years after forests were planted. In their studies of anthropogenic soil formation in Scotland, Simpson et al. (1997) interpreted $\delta^{13}C$ values of SOM in anthropogenically formed topsoils between the late 1200s and late 1800s as indicative for the application of animal manure and seaweed. Furthermore, Simpson et al. (1998) interpreted $\delta^{13}C$ values of soil organic matter in anthropogenic horizons formed in the Bronce age as indicative for the application of mainly terrestrial material with a small amount of marine-derived material. However, $\delta^{15}N$ and $\delta^{13}C$ isotope ratios of bulk soil are influenced by various processes (Balesdent and Mariotti, 1996; Boutton, 1996; Glaser, 2005; Högberg, 1997) which can limit their validity.

More specific information than by analyses of stable isotope ratios in bulk samples can be obtained by the analyses of molecular markers that are characteristic for organic material deposited by human activity. The analyses of these subsets of biomarkers can be combined with stable isotope analyses via compound-specific stable isotope analyses. For the investigation of the application of plant material Simpson et al. (1998, 1999a) used analyses of *n*-alkanes, *n*-alkanols, *n*-alkanoic acids and compound-specific $\delta^{13}C$ analyses in *n*-alkanes and *n*-alkanoic acids. With these analyses they showed that the application of terrestrial plant material, in particular grassy turf material, contributed to the formation of an anthropogenic horizon that was associated with Bronze Age cultural landscape activity in Scotland. Information about the deposition of burned plant material (ash) can be obtained by analyses of the frequently associated deposition of incomplete burned/charred biomass. Analyses of benzene polycarboxylic acids as specific markers for black carbon (biochar) is particularly suited to detect and quantify charred biomass in soils (Brodowski et al., 2005; Glaser et al., 1998, 2000, 2003, 2004a). Recently, also the applicability of n-alkane analysis and direct analysis of specific aromatic hydrocarbons was investigated for such purposes (Eckmeier and Wiesenberg, 2009; Wiesenberg et al., 2009).

Furthermore, the deposition of animal debris in archaeological context has been investigated by biomarker and compound-specific stable isotope analyses. The input of marine and terrestrial animal remains in soils at archaeological sites in northern Canada was investigated by analyses of fatty acids and steroids (Morgan et al., 1983; Nolin et al., 1994).

Fertilization with farmyard manure has been shown to influence the $\delta^{15}N$ values of amino acids in soil (Bol et al., 1997; Simpson et al., 1997, 1999a). In soils of manured grasslands, hydrophobic amino acids had higher $\delta^{15}N$ values in comparison to grasslands that had not been manured (Simpson et al., 1997, 1999a). Data from long-term experiments, from relict medieval to early modern soils and from fossil Bronze Age soils indicate that land use-induced changes in $\delta^{15}N$ values of amino acids remains stable in soil over long periods and can be used to analyse ancient fertilization with faeces (Bol et al., 2004; Simpson et al., 1997, 1999a). However, up to now, faecal deposition to soils and terrestrial sediments has been traced mainly by 5β-stanols (Bethel et al., 1994; Bull et al., 2001; Hjulström and Isaksson, 2009; Simpson et al., 1998; Sweetwood et al., 2009). The potential of 5β-stanols as biomarkers to detect sewage pollution was intensively investigated in marine and limnological environments (Bull et al., 2002; Hagedorn and Weisberg, 2009; Vivian, 1986; Walker et al., 1982). Additionally, their potential was described to identify modern faeces application in agricultural soils (Ibanez et al., 2000; Jardé et al., 2007, 2009; Puglisi et al., 2003; Rogge et al., 2006). Their high stability also permits their application in an archaeological context (Bull et al., 1999b, 2002; Evershed and Bethell, 1996). Thus, they are used to detect faecal input into archaeological soils and sediments (Bull et al., 2001, 2003; Hjulström and Isaksson, 2009; Simpson et al., 1999b; Sweetwood et al., 2009). 5β-Stanones are assumed to have a potential as biomarkers comparable with 5β-stanols (Grimalt et al., 1990) but were rarely analysed in soils and terrestrial sediments (Knights et al., 1983).

Both 5β-stanols and 5β-stanones are not exclusively attributed to anthropogenic faeces, livestock breeding or manuring. Small amounts of these substances in soils could originate from faeces of wildlife and in situ reduction of Δ^5-sterols in soils (Bethel et al., 1994). To avoid misinterpretation of the concentrations of 5β-stanols and 5β-stanones, their amounts should be related to the amounts of their precursors, Δ^5-sterols, and/or their 5α-isomers (the main reduction products of Δ^5-sterols in the environment) (Bull et al., 1999b, 2002). Thus, methods for faecal biomarker analysis should also allow quantification of Δ^5-sterols and 5α-stanols and/or 5α-stanones.

Although even small concentrations of 5β-stanols and/or 5β-stanones, if related to the concentrations of their precursors and 5α-isomers, allow reliable detection of enhanced faeces deposition, they provide only raw information about the origin of the faecal material (Bethel et al., 1994; Bull et al., 1999b, 2002; Evershed and Bethell, 1996; Leeming et al., 1997). Therefore, bile acids were analysed in some studies as additional biomarkers (Bull et al., 1999a, 2003; Knights et al., 1983; Simpson et al., 1999b) which deliver more detailed information about the source of faeces (Bull et al., 2002; Elhmmali et al., 1997; Evershed and Bethell, 1996; Tyagi et al., 2008). However, if solely bile acids are analysed, validation is not possible if small amounts of bile acids originate from enhanced faeces deposition or originate from natural background. This is hindered because no biomarker ratios for bile acids have been described which enable detection of enhanced faeces deposition analogous to the ratios of 5β-stanols and 5β-stanones to Δ^5-sterols, and/or their 5α-isomers. Thus, a reliable detection of enhanced faeces deposition in environmental samples combined with identification of the source of faeces demands at least the analyses of stanols and bile acids (Bull et al., 1999b, 2002; Evershed and Bethell, 1996).

1.2 Objectives

Knowledge about the deposition of nutrient-rich material through pre-Columbian land use is crucial for understanding intensity of pre-Columbian land use and population densities in Amazonia before the Conquest. In addition, such knowledge is necessary to develop sustainable agriculture in the humid tropics based on pre-Columbian land use techniques. The objective of this thesis is to investigate anthropogenic nutrient inputs into soils which were modified by pre-Columbian land use. For this reason, *terras pretas* and the vestiges of raised fields were analysed.

Terras pretas are characterized by high nutrients contents. Therefore, analyses of *terras pretas* should have a high potential to deliver information about the source of nutrients in these soils and consequently, about the materials which were deposited. However, it is not known if the incorporation of nutrients in these soils was the result of intentional fertilization or by chance the result of habitation. The analyses of the remains of raised-field agriculture can deliver information about intentional fertilization of agricultural fields with material from external sources by pre-Columbian farmers in Amazonia.

In this thesis, existing knowledge and hypotheses were reviewed for *terras pretas* and raised fields in Amazonia. Nutrient analyses and biomarker analyses were performed and analysis techniques were compared and developed. In the following, contents of individual chapters of this thesis are briefly summarized.

Chapter 2

In this review the knowledge about nutrients and nutrient sources in *terras pretas* is summarized and discussed in the context of their properties and their genesis.

Chapter 3

It has often been hypothesized that faeces had been a nutrient source in *terras pretas* (Glaser, 2007; Glaser et al., 2004a; Smith, 1980; Sombroek, 1966; Woods, 2003b), but no study has focussed on this question yet. In this chapter, the biomarkers for faeces 5β-stanols as well as their precursors and their 5α-isomers in *terras pretas* and reference soils were analysed to investigate the input of faeces into *terras pretas*.

Chapter 4

The high potential of simultaneous analyses of faecal biomarkers from different steroid groups is well described (Bull et al., 1999b, 2002; Evershed and Bethell, 1996). However, neither the development of a specific method for combined analyses of faecal biomarkers of different substance classes in soils or terrestrial sediments, nor detailed validation of the methods applied to such samples have been published, yet. Therefore, the aim of this study was to develop a method enabling the quantification of 5β-stanols, 5β-stanones and their 5α-isomers together with Δ^5-sterols and bile acids based on a single lipid extraction. Effects of saponification of the total lipid extract were analysed. The method was validated by standard addition to the total lipid extract from samples of six different soils, including a *terra preta* and other Anthrosols. The standard contained 5β-stanols, 5 β-stanones and their 5α-isomers together with Δ^5-sterols and bile acids (19 substances).

Chapter 5

In this chapter, ideas about the management of nutrients in pre-Columbian raised-field agriculture in Meso- and South America were summarized in the context of the functioning of this agricultural technique. The management of nutrients in analogous outside of Meso- and South America is reviewed. Hypothesis how nutrients were managed by pre-Columbian raised-field farmers in Amazonia and other areas of Meso- and South America were developed.

Chapter 6

This chapter presents a study which aims to analyse if the physical legacy of persistent raised fields in French Guiana is accompanied by a soil chemical legacy of higher soil organic matter and nutrient concentrations, and if fertilization with material from an external source can be proved. For this purpose, raised fields belonging to all types of mounds and ridges previously described in French Guiana (McKey et al., 2010; Rostain, 2008a, 2010) were sampled at five sites and their properties compared to adjacent soils in flat savanna areas. Total organic carbon and total nitrogen were analysed. Plant-available nutrient concentrations were analysed and compared with pseudo-total nutrient concentrations at two sites.

Chapter 2

State of the scientific knowledge on properties and genesis of Anthropogenic Dark Earths in Central Amazonia (*terra preta de Índio*)

Glaser B. and Birk J.J., 2012.

Geochimica et Cosmochimica Acta 82, 39–51.

2.1 Abstract

Tropical rainforests are highly important for the global climate regulation and for global biodiversity. However, these ecosystems are characterized by nutrient-poor and highly weathered soils and by high turnover rates of organic matter. Thus, they are fragile ecosystems prone to loss of ecosystem services when anthropogenically disturbed. Currently, the major threat to these ecosystems is deforestation leading to irreversible destruction of rainforests. Surprising and not expected is that within these ecosystems small patches of highly fertile soils occur which are known as Anthropogenic Dark Earths or *terra preta de Índio* (*terra preta*) These soils exhibit high nutrient and soil organic matter stocks and allow sustainable agriculture. Frequent occurrence of pot-sherds of pre-Columbian origin and further evidence for settlement activities clearly demonstrate that *terra preta* is of anthropogenic origin. In recent years, the *terra preta* phenomenon has gained increasing interest because it is assumed that *terra preta* could act as a model for promoting sustainable agricultural practices in the humid tropics and because *terra preta* is an example for long-term CO_2 sequestration into terrestrial ecosystems with additional positive benefits for ecosystem services. These potentials of *terra preta* initiated a great number of studies but also stimulated fantasy about their genesis. Therefore, the aim of this review is to summarize the scientific knowledge about *terra preta* properties and to discuss their genesis. From our own and literature data it is evident that *terra preta* is the product of inorganic [e.g. ash, bones (esp. fish)] and organic (e.g. biomass wastes, manure, excrements, urine, and biochar) amendments to infertile Ferralsols. These ingredients were microbially metabolized and stabilized by humification in soil, fungi playing a bigger role in this process compared to bacteria in surrounding ecosystems. Biochar is a key component for this process due to its stability and its enrichment in *terra preta*. It is still unclear if *terra preta* was produced intentionally or un-intentionally. In addition, it is unclear how much time was needed after the disposal of the materials mentioned above to develop a *terra preta*. Further research is highly desired to investigate these latter two issues.

2.2 Introduction

Protection of rainforests and development of sustainable land use in the humid tropics is of global significance because rainforests in the humid tropics exhibit a high biodiversity and are of high importance for the regulation of Earth's climate (Bonan, 2008; Lewis, 2006; Trumper et al., 2009).

For many years, it has been assumed that environmental limitations inhibit intensive agriculture in Amazonia (Meggers, 1954). Amazonian ecosystems are characterized by low fertility of the predominant, highly weathered soils. Extensive shifting-cultivation was the most adopted agriculture in these ecosystems during recent centuries (Kleinman et al., 1995). In modern times, increasing population pressure caused expansion of the area under cultivation, as well as reduced regeneration times followed by soil degradation (IPCC, 2000; Fearnside, 2005). Deforestation was boosted by further overexploitation, especially large-scale cattle raising (Fearnside, 2005). Currently, these ecosystems are rapidly destroyed as a consequence of anthropogenic overexploitation (Fearnside, 2005; Lewis, 2006; Trumper et al., 2009). Intensive human land use in Amazonia – now and in the recent past – would always lead to soil degradation (McKey et al., 2010).

Patches of dark-coloured soils have been found in Amazonia (Fig. 2.1). They are known as Anthropogenic Dark Earths (ADE) or *terra preta* (*de Índio*) and they exhibit completely different properties than most soils in this region (Fig. 2.1). These soils are characterized by large stocks of stable soil organic matter (SOM) and high nutrient levels (Glaser, 2007; Glaser et al., 2001b). After pedological and archaeological investigations, predominantly over the last few decades, the anthropogenic origin of ADE is no longer questioned (Glaser, 2007; Glaser et al., 2004c; Neves et al., 2003; Woods and Denevan, 2009).

Archaeological evidence and radiocarbon dating showed that they were formed by pre-Columbian inhabitants, although *terra preta* formation was a more variable and sometimes faster process than previously thought (Heckenberger et al., 2003; Neves et al., 2003). Radiocarbon dates for *terra preta* sites at the Upper Xingu region and in Central Amazonia ranged between 60 and 1640 AD (Heckenberger et al., 2003) and 2500 and 500 a BP (Neves et al., 2003), respectively.

Amazonian Dark Earths could act as a model for sustainable agriculture in the humid tropics (Glaser, 2007; Glaser et al., 2001b; Sombroek et al., 2002) and for other soils which exhibit a low nutrient holding capacity. Agricultural techniques leading to *terra preta* formation have the potential to stop increasing land degradation from recently employed intensive agriculture in these regions and to reclaim degraded areas (Glaser, 2007). Additionally, the high stability of SOM in ADE (Glaser, 2007) and experimental data (e.g. Kuzyakov et al., 2009) show that in principle, carbon can be sequestered for millennia in soils. So regeneration of *terra preta* has the potential to combine *terra preta* sustainable agriculture with long-term CO_2 sequestration.

The existence of ADE has been known for more than 100 years, but only in the last decade has it attracted broad scientific interest (Woods and Denevan, 2009). The potential of ADE not only motivated research, but also stimulated a huge number of hypotheses and ideas concerning their genesis, partly hurrying ahead of the scientific knowledge. Therefore, the aim of this review is to summarize the scientific knowledge about *terra preta*, focussing on properties and genesis.

Figure 2.1 Left: Typical Ferralsol profile. The shallow surface horizon is light brown coloured and roots are concentrated at the soil surface. Bellow this horizon follows a thin transition horizon to a subsoil horizon which can be several meters thick. The texture of these soils is loamy or sandy and the structure is dominated by stable micro aggregates (pseudo-sand). Right: typical *terra preta* profile. The topsoil horizons are dark grey or black coloured and can reach a depth of more than 1 m. Potsherds, small bone and charcoal particles are characteristic for this horizon. Roots reach deeper down in higher density than in Ferralsols and signs of bioturbation and aggregates of biogenic origin can be found frequently. Below follow transition horizons which are lighter coloured and typically show patches of different brown, grey and black colours with clear signs of mixing of topsoil and subsoil material. The subsoil horizons are identical to the subsoil horizons of adjanced soils. *Terras pretas* typically have the same texture like surrounding soils (from Glaser et al., 2001b).

2.3 Classification and occrence of ADE

2.3.1 Classification

According to the World References Base of Soil Classification, *terra preta* has been classified as hortic, terric or plaggic Anthrosols (Kämpf et al., 2003; Sombroek et al., 2002; Teixeira and Martins, 2003). However, a unique classification is still missing (Kämpf et al., 2003; Sombroek et al., 2002). To define *terra preta* is complicated by great variability of different sites as well as within single sites. Such differences are caused by differences in occupation time and land use structure. Some authors differentiate among types of dark coloured Anthrosols in Amazonia (e.g. *terra mulata* vs. *terra preta*) to describe soils showing intermediate properties between *terra preta* and surrounding soils (McCann et al., 2001; Sombroek, 1966; Woods and McCann, 1999). Only limited data from these intermediate soils exist. Therefore, this review focuses on typical *terra preta*, which is roughly defined as a soil characterized by a several decimeter-thick topsoil horizon with high levels of SOM, biochar, and nutrients (especially phosphorus) and which contains archaeological artefacts of pre-Columbian origin.

2.3.2 Occurrence

Terra preta sites occur throughout Amazonia (Denevan, 1996; Glaser, 2007; Kern et al., 2003; McCann et al., 2001; Smith, 1980; Sombroek, 1966; Sombroek et al., 2002; Woods and McCann, 1999; Fig. 2.2). Accumulation of *terra preta* sites were found in Central Amazonia and in the Upper Xingu region in Brazil, but they occur also in Amazon parts of Peru, Columbia and southern Venezuela and in the Guianas, but with lower density (Denevan, 1996; Heckenberger et al., 2003; Kern et al., 2003; Sombroek et al., 2002).

Only vague estimations exist about their total extension, ranging up to 10% of Amazonia (Mann, 2002). However, survey in southeastern Amazonia and northeastern Rondonia revealed an overall spatial coverage of one Amazonian Dark Earth per 2 km^2 (Sombroek et al., 2002). Therefore, the real extent of *terra preta* in Central Amazonia might well be underestimated.

Terra preta can be found in all eco-regions and landscapes of Amazonia (Kern et al., 2003). Most *terra preta* sites have been found near whitewater rivers (Kern et al., 2003). But they also occur also along clearwater and blackwater rivers and in upper catchment areas (Kern et al., 2003; Smith, 1980; Sombroek et al., 2002). They are generally located on non-floodable land (*terra firme*) in strategically advantageous topographic positions

(Kern et al., 2003; Fig. 2.3). However, Teixeira et al. (2008) mentioned that *terra preta* also occurred in floodplains, covered by sediments or destroyed by lateral movements of rivers. Typically, *terra preta* is developed from Ferralsols (Fig. 2.1) and Acrisols, but it is also described on a variety of other type of soils including Arensols, Podzols, Luvisols, Nitisols, and Cambisols (Kern et al., 2003).

Patches of *terra preta* are described ranging in size from less than a hectare up to several square kilometers (Kern et al., 2003; McCann et al., 2001; Sombroek et al., 2002; Woods and McCann, 1999). Large *terra preta* sites occur near major rivers, while in upper catchment areas only smaller *terra preta* sites were found (Kern et al., 2003; Smith, 1980; Sombroek et al., 2002). It seems logical that large pre-Columbian settlements were located near rivers but it is not clear if they have only been discovered at these easily accessible locations and if they also exist in areas farther away from rivers.

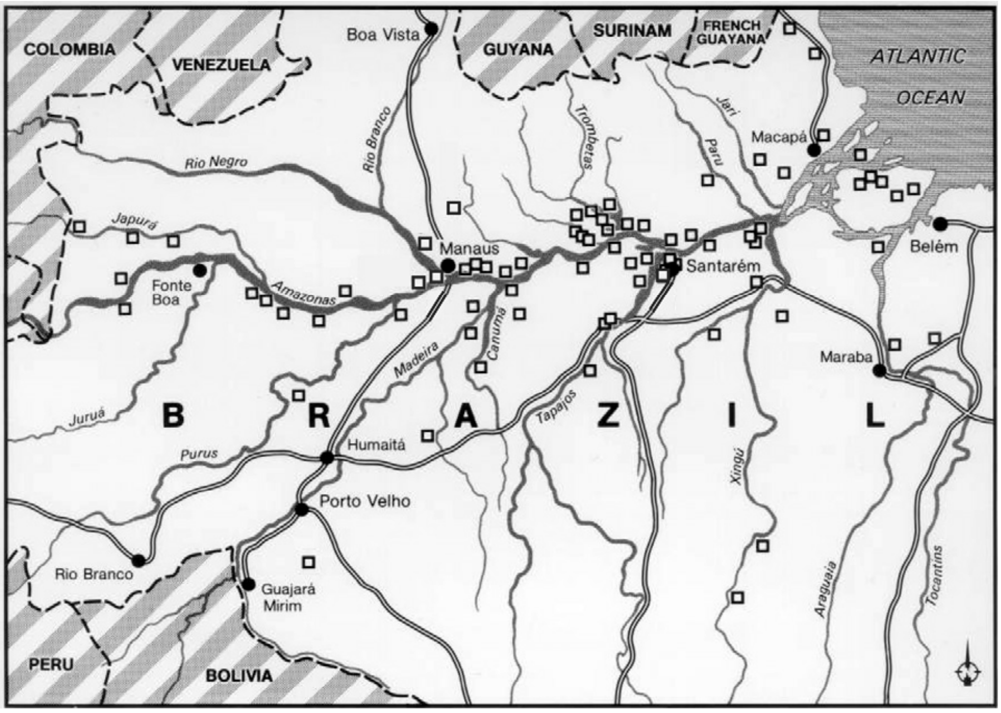

Figure 2.2 Known *terra preta* sites in Central Amazonia (Glaser, 2007; modified).

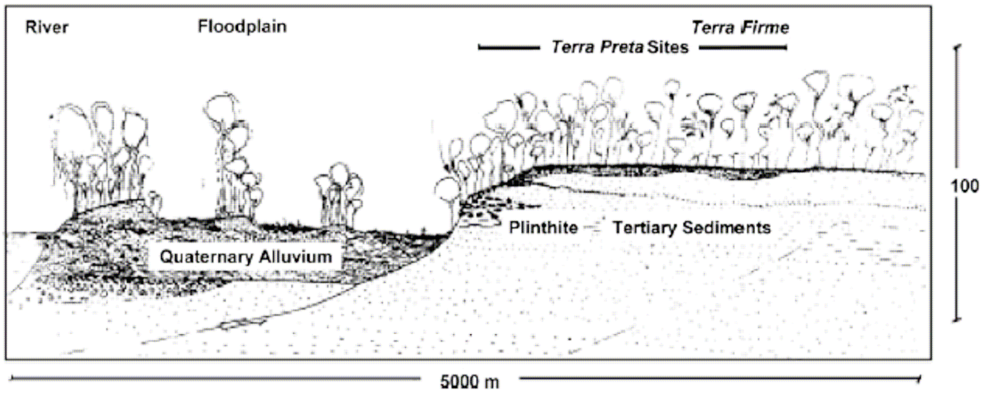

Figure 2.3 Typical location of *terra preta* sites at the non-flooded *terra firme* close to the floodplain of large rivers (Lima et al., 2002; modified).

2.4 Ecological properties of *terra preta*

2.4.1 pH

Naturally occurring Ferralsols, Acrisols, and Arenosols have a high acidity (~pH 4; Fig. 2.4) causing high levels of exchangeable Al (Fig. 2.4) and thus Al toxicity in plants. *Terra preta* is less acidic, pH values ranging from 5.2 to 6.4 (Falcão et al., 2009; Fig. 2.4). Therefore, plants grown on *terra preta* do not have Al toxicity problems, and this must be one reason for their high productivity (Falcão et al., 2009).

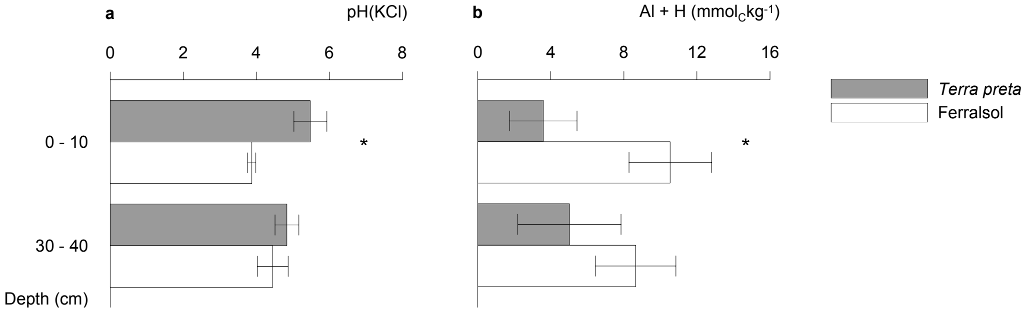

Figure 2.4 (a) pH and (b) exchangeable acidity among five *terra preta* sites near Manaus and Santarém and adjanced Ferralsols [data from Glaser et al. (2004c); mean and standard errors; * indicate significant differences ($P < 0.05$) between *terras pretas* and Ferralsols in pair-wise comparisons].

2.4.2 Nutrients in terra preta

Naturally occurring Ferralsols, Acrisols, and Arenosols contain very low amounts of most nutrients. In contrast, *terra preta* is characterized by high levels of P, N, and basic nutrients. Nutrient stocks in *terra preta* are several times greater than those of surrounding soils (Costa and Kern, 1999; Lehmann et al., 2003a; Fig. 2.5).

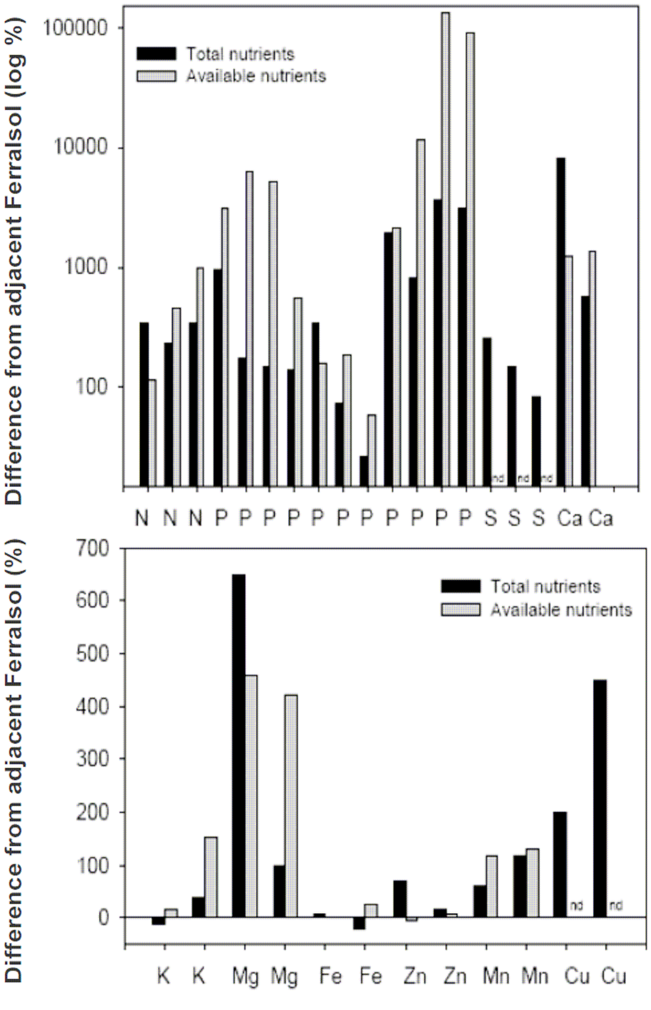

Figure 2.5 Plant-available and total nutrient contents of several *terra preta* sites in comparison to adjanced Ferralsols: Difference (%) = (Nutrient content$_{terra\ preta}$ - Nutrient content$_{\mathrm{Ferralsol}}$)/(Nutrient content$_{\mathrm{Ferralsol}}$) * 100 (%). Note differences in scale between the two graphs (Lehmann et al., 2003a; modified).

Total, P, Ca, and Mg are enriched multiple times in *terra preta* relative to surrounding soils and amounts of K, Zn, and Mg were also found at higher levels in *terra preta* (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b; Lehmann et al., 2003a; Sombroek, 1966; Woods and McCann, 1999; Zech et al., 1990; Fig. 2.5).

However, *terra preta* does not necessarily have high availability of all nutrients important for plant growth (Lehmann et al., 2003a). Generally, *terra preta* contains >200 mg kg^{-1} of plant-available P, in contrast to surrounding soils which contain about 5 mg kg^{-1} (Falcão et al., 2009). Also plant-available Ca is strongly enriched in relation to surrounding soils, but plant-available K, Mg, Fe, and N are not necessarily higher (Lehmann et al., 2003a; Fig. 2.5).

Therefore, often reported higher crop yields on *terra preta* depend on crop species; e.g. crops with high K requirements may not grow as well (Falcão et al., 2009; Lehmann et al., 2003a) as crops with high P requirements (Lehmann et al., 2003a). The low concentrations of plantavailable K combined with high concentrations of Ca constitute an imbalanced nutritional status for many crops (Falcão et al., 2009).

2.4.3 Nutrient holding capacity

In naturally occurring soils of Central Amazonia, low activity clays such as kaolinite predominate in highly weathered Ferralsols and Acrisols, while quartz being the dominating mineral in Arenosols. Both kaolinite and quartz have an extremely low capacity to hold nutrients (normally measured as cation exchange capacity, CEC) being lower than 12 cmol$_c$ kg^{-1}. Therefore, SOM plays a major role in the nutrient cycle providing sites for cation exchange. However, due to unfavourable climatic conditions (heavy rainfall and high temperatures) SOM levels of Amazonian soils are normally low because of high mineralization rates. For these reasons, naturally occurring upland Amazonian soils have low nutrient holding capacity. This is the reason why slash-and-burn or fertilization with commercial (NPK) fertilizer is inefficient and not sustainable.

Being rich in SOM, *terra preta* provides a high CEC of 13–25 cmol$_c$ kg^{-1} (Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b, 2003; Lehmann et al., 2003a; Sombroek, 1966; Zech et al., 1990; Fig. 2.6), preventing nutrients from leaching. Clay mineral analyses showed a similar mineral composition of *terra preta* and reference soils, dominated by kaolinite and accessory minerals such as goethite, hematite, anatase (Bechthold, 1982). Correlation of CEC with SOM concentrations shows that SOM is primarily responsible for CEC of *terra preta* as it is naturally occurring in Amazonian soils (Sombroek, 1966). However, *terra preta* has on average three times greater SOM content than surrounding soils (Glaser, 2007; Fig. 2.6). Not only is the quantity of SOM responsible for the high CEC, but so is SOM quality. ^{13}C NMR (Zech et al., 1990) and DRIFT spectra (Cunha et al., 2009) revealed that SOM in *terra preta* contains higher amounts of carboxylic groups and phenolic groups compared to surrounding soils. For these reasons, SOM in *terra preta* has higher CEC than SOM in naturally occurring soils (Liang et al., 2006; Sombroek, 1966; Fig. 2.7).

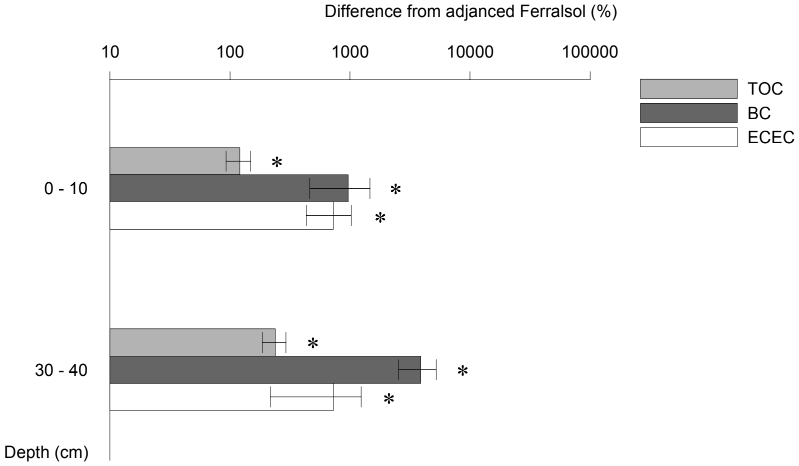

Figure 2.6 Cation exchange capacity at soil pH (ECEC), total organic C (TOC) and biochar (BC) concentration of five *terra preta* sites near Manaus and Santarém in comparison to adjanced Ferralsols: Difference (%) = (Value$_{terra\ preta}$ - Value$_{\text{Ferralsol}}$)/(Value$_{\text{Ferralsol}}$) * 100 (%) [data from Glaser et al. (2004c); mean and standard errors; * indicate significant differences (P < 0.05) between *terras pretas* and Ferralsols in pair-wise comparisons].

2.4.4 SOM stability

In the humid tropics, a combination of high temperatures and a favourable soil moisture regime induces high rates of SOM decomposition and weathering of primary minerals (Sombroek et al., 1993; Tiessen et al., 1994). For these reasons, naturally occurring Amazonian soils have a low nutrient-holding capacity.

During a laboratory incubation experiment, Glaser (1999) found that *terra preta* under cultivation contained greater amounts of rapidly mineralizable and very stable SOM (passive/recalcitrant) both in absolute terms and as fraction of the total SOM compared to surrounding soils.

Analysis of carbohydrates and lignin suggests that an enhanced input of unaltered plant material is responsible for the higher amounts of labile SOM in *terra preta* under cultivation. Mineralization of labile SOM contributes to the high amounts of plant-

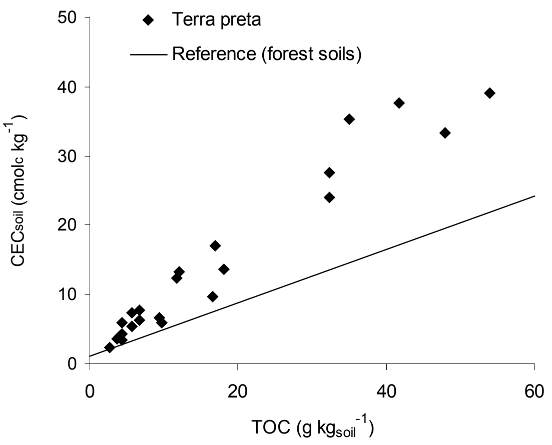

Figure 2.7 Correlation between humus content (measured as total organic carbon, TOC) and nutrient holding capacity (measured as potential cation exchange capacity with ammonium acetate buffered at pH 7) of *terra preta* and surrounding soils. Note that the slope of *terra preta* is higher by a factor of 3 compared to surrounding soils which means that the SOM quality of *terra preta* is much better with respect to the ability to prevent nutrients from leaching (redrawn after Sombroek et al., 1993).

available nutrients in *terra preta* (Glaser, 1999), indicating that nutrient cycling in these soils is intimately related to the SOM because of the low supply of weatherable minerals.

Besides this labile SOM pool, *terra preta* contains a higher stable SOM pool than surrounding soils which can be explained by physical and organo-mineral stabilisation and also by higher recalcitrance against microbial degradation (Glaser et al., 2003). A higher physical stabilization via inclusion into the interior of aggregates was found in ADE (20%) compared to adjacent soils (10%) (Glaser, 1999). Thus, there is proportionally more SOM stabilized in aggregates in *terra preta* compared to adjacent sites. Besides a physical stabilization of SOM, density and particle size fractionations revealed that a major part of SOM in *terra preta* is stabilized by interaction with soil minerals (Glaser et al., 2003). As kaolinite is the dominating clay mineral having a low interaction potential, it is most likely that interaction occurs at the surface of iron and aluminium oxides. Analytical data further showed that the main reason for the high stability is the chemical recalcitrance of SOM in *terra preta*. ^{13}C NMR spectra revealed that SOM in *terra preta* is rich in condensed aromatic structures (Glaser et al., 2003; Novotny et al., 2009; Zech et al., 1990). Diffuse-reflectance infrared Fourier transformation (DRIFT) spectrometry, electron paramagnetic resonance (EPR) spectroscopic analyses showed that humic acids in *terra preta* contain more aromatic structures being more condensed than humic acids in reference soils (Cunha et al., 2009).

Benzenepolycarboxylic acids (BPCA) is a molecular marker, but it is not a “natural” marker. It is formed when biochar is treated with nitric acid. It is used as an index of the amount of char in a sample. Using BPCA as molecular markers, it has been shown that the condensed aromatic structures originated from incomplete combustion (Glaser et al., 1998, 2003). These structures were called black carbon containing biochar but also other components such as soot (Glaser, 2007). Five *terra preta* sites in Central Amazonia contained a mean of about 50,000 kg biochar per hectare and one meter soil depth, being enriched by a factor of 70 compared to adjacent soils (Glaser et al., 2001b).

It is believed that biochar persists in the environment over millennia due to its biological and chemical recalcitrance caused by the polyaromatic backbone (Goldberg, 1985; Schmidt et al., 1999). The existence of *terra preta* even today proves that biochar is stable over millennia in extreme environments such as the humid tropics. Using ^{14}C-labelled biochar, Kuzyakov et al. (2009) calculated a mean residence time of biochar to be about 2000 years, although there is clearly a continuum of degradability that is related to the temperature and duration of heating during biochar formation. Nevertheless, biochar is a key factor for the stability of the SOM in *terra preta* (Glaser, 1999; Glaser et al., 2000, 2001b) and thus, for the *terra preta* genesis itself.

^{13}C NMR spectroscopy further revealed a signal corresponding to aromatic acids such as mellitic acid (Möller et al., 2000), which is considered being an end product of biochar oxidation (Glaser et al., 1998, 2001b). Thus, slow biochar oxidation over time produced carboxylic groups on the edges of the aromatic backbone, which increased the nutrient retention capacity (Glaser et al., 2000). From these results it can be concluded that biochar found in *terra preta* is not only responsible for the long-term SOM stability evident today, but is also a direct key factor for the high CEC in *terra preta* (Glaser 2007; Glaser et al., 2003).

2.5 Theories of *terra preta* genesis

2.5.1 Sedimentation of allochthonous soil material vs. in situ genesis

Terra preta research began in the 1870s and throughout the publications of the 19th and early 20th centuries, all authors (Hartt, Orton, Smith, Derby, Katzer) recognized that these soils were anthropogenic and clearly stated so. It was not until the 1940s that we start to get references to geogenic origins in the literature (Woods and Denevan, 2009).

For instance, volcanic (Hilbert, 1968) and fluvial (Franco, 1962; Zimmermann, 1958) sedimentation were suggested. With the current pedological knowledge that *terra preta* and the surrounding soils have a similar mineralogical composition and texture, all geogenic hypotheses of *terra preta* formation can be rejected. For instance, upon volcanic sedimentation one would expect the deposition of tephra layers coupled with a different particle-size distribution, the occurrence of volcanic glasses and their weathering products (allophanes), and different heavy minerals (Gillespie et al., 1992; Zech et al., 1996) in *terra preta*. A fluvial sedimentation would also result in a different texture. However, such observations have never been reported (Glaser et al., 2002; Sombroek et al., 1993; Zech et al., 1979).

It is often noted that *terra preta* developed on extensive soils in Amazonia: Ferralsols, Acrisols, and Arenosols. They have also developed from Plinthosols, Cambisols and other types of soils (Kämpf et al., 2003). Due to similar mineral composition of *terra preta* and adjacent sites and an even integration of *terra preta* into the surrounding landscape, a genesis from anthropogenically applied allochthonous soil material is also excluded. Therefore, it is more likely that *terra preta* formed in situ by addition of biochar and nutrient-rich waste materials.

2.5.2 Sources of biochar

The question of how the large amounts of biochar accumulated in *terra preta* has been only partly answered, because it is difficult to distinguish between naturally occurring and anthropogenic biochar formation (Glaser et al., 2004a). Commonly it is assumed that biochar is a pyrogenic material which is the residue of incomplete burning of biomass (charcoal, black carbon). Glaser et al. (2001b) calculated that a total of approximately 25 forest burnings would be necessary to accumulate the mean biochar content found in *terra preta* (50,000 kg per hectare). Therefore, exclusively naturally formed biochar as residue after forest fires could be responsible for the *terra preta* formation. However, as naturally occurring forest fires are widespread in Amazonia, one can assume that over periods reported for the *terra preta* formation of up to 3000 years, the whole of Amazonia should be influenced to more or less the same extent. If true, Amazonia should be covered entirely by *terra preta*, which is certainly not the case.

Terra preta does not form in soils under shifting cultivation or slash and burn (Woods and McCann, 1999), strongly suggesting that biochar accumulation into *terra preta* was not due to natural forest fires. Repeated slash-and-burn of abandoned settlement sites, however, could have produced *terra preta* as outlined above (Glaser, 1999). A number of other anthropogenic activities could also be responsible for biochar accumulation and subsequent formation of *terra preta*, such as the use of low heat, smouldering fires for food and pottery preparation, spiritual reasons, or biochar amendments to home gardens (Glaser et al., 2001b).

Recently, indigenous groups as well as *caboclos* (people of mixed origin) are using various forms of *terra quemada* or very similar soil conditioner in home gardens which could have contributed to the high biochar levels in *terra preta* if also done during pre-Columbian times. *Caboclos* are sweeping their home gardens and burning or charring the debris before applying to home gardens e.g. around tree seedlings and young trees (WinklerPrins, 2009). Another source of biochar could be heating (charring) of soil by hot fire in home gardens as practiced by an indigenous group near Manaus (Steiner et al., 2009). At the border between Brazil and French Guiana, *caboclos* collect wall material from charcoal kilns consisting of mixed charcoal and heated soil and mix it with manure for seed germination in pots even today. Some people in Brazil cultivate plants in elevated gardens containing high biochar concentration.

2.5.3 Nutrient sources and further amendments

With respect to potential sources for the high nutrient stocks, in situ weathering as a source of P, Mg, K, Zn, and Mn can be excluded, at least for heavily weathered Ferralsols and Acrisols and poorly developed infertile Arenosols, since these soils do not contain high concentrations of these elements (Costa and Kern, 1999; Cunha et al., 2009; Glaser, 2007; Glaser et al., 2001b; Lehmann et al., 2003a; Sombroek, 1966; Zech et al., 1990). Only N can be induced biologically in situ via N fixation (Glaser, 2007). Other elements must be incorporated from the surroundings for nutrient accumulation (Glaser, 2007). Mapping of element concentrations of *terra preta* sites showed different distribution patterns of elements indicating different nutrient sources (Costa and Kern, 1999). As pre-Columbian populations had no access to mineral fertilizers such as NPK, only local resources can be responsible for nutrient accumulation in *terra preta*. The following sources could be identified unambiguously. As biochar contains only traces of nutrients, it does not significantly contribute to the nutrient status (Glaser, 2007). Arroyo-Kalin et al. (2009) and Woods (2003b) indicated that ash may have been a significant input into *terra preta*. It is likely that ash was applied on *terra preta* by human activities. However, it is unlikely that the application of ash was a key process leading to *terra preta* formation as slash-and-burn agriculture does not generate *terra preta* despite the fact that a lot of ash is applied to such sites. In addition, ash contains significant amounts of Ca, K, Mg, and P, while *terra preta* is highly enriched in P, but other elements are less enriched or even depleted, especially K (Fig. 2.5). Therefore, the quantities and ratios of nutrients in *terra preta* indicate that plant materials were not the only nutrient sources.

It has been hypothesised that plant biomass from rivers and organic matter from floodplains were transported to the sites (Denevan, 1996; Glaser, 2007; Lima et al., 2002). Here again, elemental ratios atypical for plants contradict the idea that decomposition of large amounts of plant biomass (not charred or burned) was a key process leading to *terra preta* formation. In addition, application of high amounts of plant biomass from rivers and organic matter from floodplains could be possible in regions of white water rivers where fertile floodplains (*várzea*) are found, but does not help explaining the nutrient stocks in numerous *terra preta* sites near blackwater rivers, which are nutrient poor and support only a floodplain characterized frequently by white sands (Prance, 1979).

Plant material originating from mulching or residues of house construction could contribute to the nutrient stocks. This is difficult to test analytically, but like ash and plant biomass from rivers and organic matter from floodplains, it is unlikely to be a key process for *terra preta* genesis. Again here, nutrient patterns in *terra preta* are not typical for plant biomass and soils under natural rain forest show a high litter production, not leading to *terra preta* formation.

Another potential nutrient source is waste, including plant residues from food production, animal remains such as mammal and fish bones which likely contribute to the nutrient stocks. Fish bones can be even observed in *terra preta* profiles today. By scanning electron microscopy in combination with energy-dispersive X-ray spectroscopy (SEM/EDS) Lima et al. (2002) and Schaefer et al. (2004) found evidence for high Ca and P derived from bones in some *terra preta*.

Last, animal manures including human waste may have contributed nutrients to *terra preta* (Glaser, 2007; Glaser et al., 2004c; Smith, 1980; Sombroek et al., 2002; Woods, 2003b). Recently, determinations of manure-specific steroids have shown that coprostanol (5ß-cholestan-3ß-ol) and epicoprostanol (5ß-cholestan-3α-ol) are the two most enriched stanols in *terra preta* compared to surrounding reference soils, indicating enhanced input of faecal material in *terra preta* (Fig. 2.8).

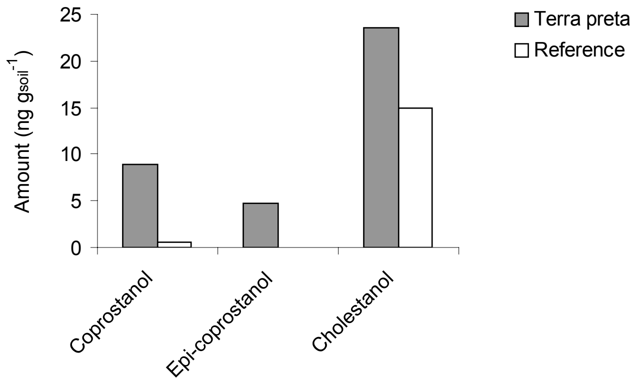

Figure 2.8 Concentrations of faecel stanols (coprostanol and epicoprostanol) and concentration of a stanol which was built in the soil (cholestanol) in a *terra preta* sampled near Santarém compared to surrounding soil (30–40 cm; Birk et al., 2010).

The identification of the type of faeces is hindered by limited data of steroid composition of Amazonian animals. In contrast to coprostanol being a biomarker for faeces of omnivores (Bethel et al., 1994; Bull et al., 1999b, 2002; Evershed et al., 1997), the stanols which are characteristic for the faeces of herbivore mammals are not enriched in *terra preta* to same extent. Thus, faeces-derived nutrient input into *terra preta* was most probably introduced by omnivores (Birk et al., 2010). For further identification of faeces-derived nutrient input into *terra preta*, analysis of bile acid pattern might help. Bile acids in a *terra preta* were dominated by deoxycholic acid accompanied by minor concentrations of lithocholic acid (Fig. 2.9), typical for faeces of humans (Bull et al., 1999b, 2002). Therefore, all available steroid data so far unambiguously indicate deposition of human faeces, which could explain the abnormal nutrient pattern dominated by large amounts of P.

The stanols observations are corroborated by compound-specific $\delta^{15}N$ analysis of individual amino acids. These analyses showed that *terra preta* exhibit more positive $\delta^{15}N$ values of hydrophobic amino acids than the control soil (Fig. 2.10), which is characteristic for soils fertilized with excrements (Simpson et al., 1997). This effect could only be detected in deeper horizons of *terra preta*. The lack of higher Δ15N values in the topsoil could be caused by recent land use, which effects a dilution of the ancient land use signal.

In conclusion, the following sources of nutrients in *terra preta* seem most likely: plant biomass, mammal and fish bones, ash, biochar, and human excrement (Fig. 2.11).

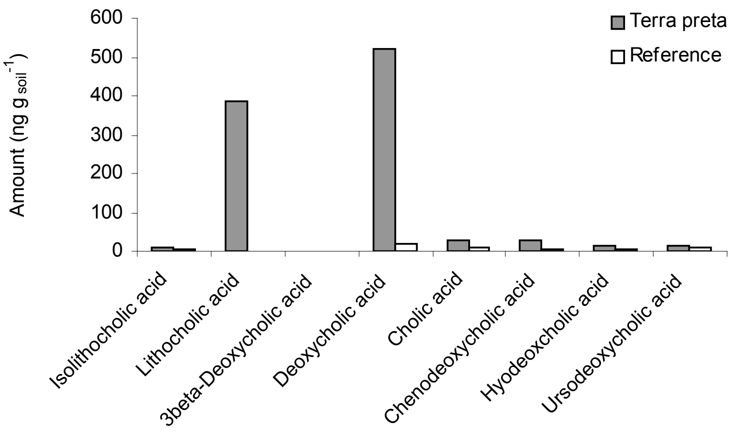

Figure 2.9 Bile acid concentrations in a *terra preta* sampled near Santarém compared to surrounding soil (0–50 cm; Birk et al., 2010).

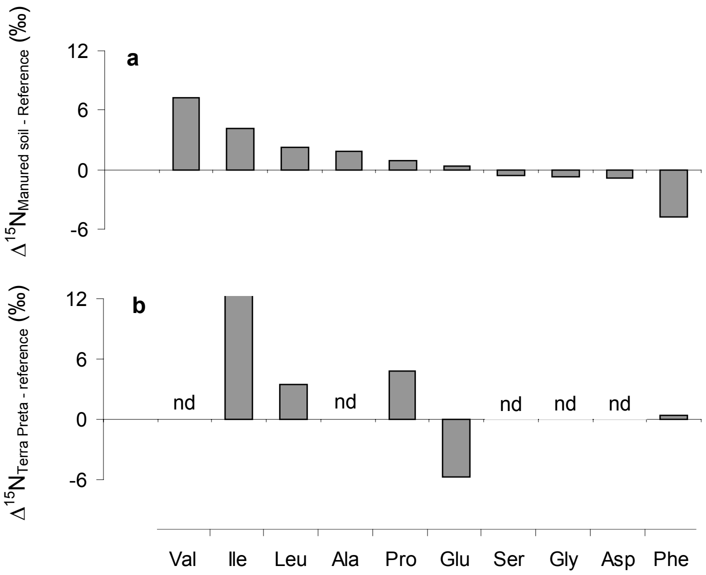

Figure 2.10 (a) Differences of $\delta^{15}N$ values of individual amino acids between a manured soil and a control soil in Europe ($\Delta^{15}N$; Simpson et al., 1997) and (b) between a *terra preta* (30–40 cm) sampled near Manaus and the surrounding soil (lower part; Birk et al., 2010).

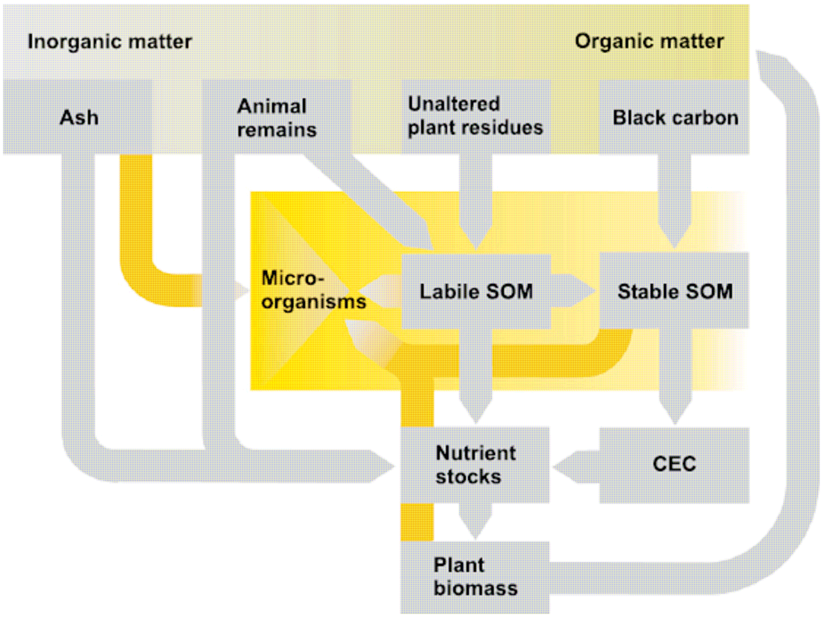

Figure 2.11 Model of *terra preta* genesis.

2.5.4 Role of microorganisms in terra preta genesis

Much discussion has been associated with the biology and specifically the microbiology in *terra preta* (Lehmann, 2009). It is assumed that microorganisms are responsible for the oxidation of biochar, providing functional groups for nutrient retention (Glaser et al., 2002), that microorganisms contribute significantly to the formation of stable SOM in *terra preta* (Glaser, 2007), or that specific microorganisms were responsible for the genesis of *terra preta* (Woods and McCann, 1999).

Recently, some studies have been focused on the microbiology of *terra preta*. These studies revealed a distinct microbial community in *terra preta*. Methods with direct microbial cultivation as well as direct DNA extractions from soils showed a higher microbial diversity and species richness in *terra preta* compared to reference soils (Kim et al., 2007; O’Neill et al., 2009; Ruivo et al., 2009; Tsai et al., 2009). It turned out that *terra preta* is rich in K-selected bacteria typical for more stable environments, with slower rates of C turnover in contrast to an abundance of r-selected bacteria in reference soils characteristic of rapid C mineralization (O’Neill et al., 2009). Cultivation experiments in different *terra preta* gave evidence for a predominance of fungal populations vs. bacterial populations (Ruivo et al., 2009).

In general, the recent literature suggests that the microbial communities in *terra preta* are a result of the unique *terra preta* habitat as opposed to contributing significantly to *terra preta* genesis. In addition, it is now clear that it will not be possible to extract a special microbial population that can be used to recreate *terra preta* (Lehmann, 2009). However, the microbial community may perform functions that change soil nutrient and carbon dynamics in ways that promote the self-perpetuation of *terra preta* (Lehmann, 2009). The latter is supported by biomarker analyses.

Cultivation experiments and direct extraction of DNA from soils deliver information about the recent microbial community composition which is not necessarily comparable to the composition during the times of *terra preta* generation. Therefore, analyses of more stable microbial biomarkers such as amino sugars and muramic acid being indicators for fungal and bacterial residues, respectively (Amelung, 2001), seems more promising with respect to reconstruction of microbial impact on *terra preta* formation. *Terra preta* showed elevated amino sugar to muramic acid ratios compared to reference soils (Fig. 2.12), indicating enhanced impact of fungi compared to bacteria (Glaser et al., 2004b). These results clearly indicate the higher importance of fungi over bacteria with respect to *terra preta* formation, although amino sugars are certainly not so stable than biochar or black carbon, they are more stable in soil than other microbial biomarkers (Glaser et al., 2004b). In addition, low microbial oxidation in deeper soil has certainly an additional conservation effect. Furthermore, due to similar chemical structure and thus stability, the ratio between glucosamine (=fungal marker) to muramic acid (=bacterial marker) is valid independent from their absolute stability.

The enhanced proportion of fungi in *terra preta* could have favourable consequences on the soil properties of *terra preta*. Recent studies have investigated whether abiotic processes or microorganisms are of greater importance in the oxidation of biochar. Hamer et al. (2004) found a close correlation between glucose mineralization and biochar mineralisation during biotic incubation for model chars, which could suggest a co-metabolic degradation of biochar by microbial oxidation. On the other hand, Cheng et al. (2006) found no differences in surface oxidation of biochar during a 30-day incubation experiment of sterilized biochar and biochar soil mixtures in comparison to these materials inoculated by a commercially available microbial inoculum. They postulated that abiotic processes were more important for oxidation of biochar than biotic processes but qualified that microbial oxidation could play an important role over long periods and that specialized microorganisms could have great influence on biochar oxidation. Such microorganisms could be fungi. The enhanced proportion of fungi in the microbial community in *terra preta* can be interpreted as a hint for a microbial oxidation of biochar.

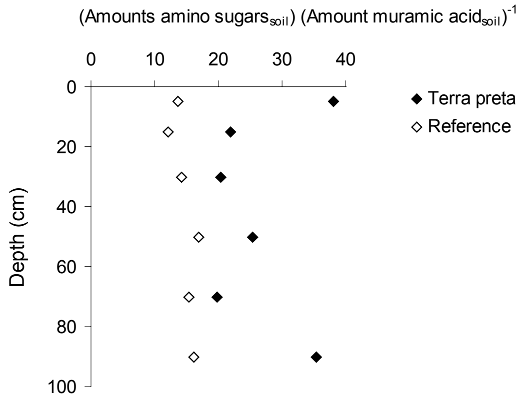

Figure 2.12 Amino sugar and muramic acid concentrations in a *terra preta* sampled near Santarém compared to an adjacent soil (Birk et al., 2010).

In addition, there is scientific evidence for biological production of black carbon in *terra preta* by fungi (Glaser and Knorr, 2008). Aspergillin, the black pigment of *Aspergillus niger* (which is ubiquitous in soils) was reported to possibly contain condensed aromatic structures (Lund et al., 1953) similar to those of black carbon or biochar (Schmidt and Noack, 2000). The contribution of aspergillin to soil black carbon contents was estimated to be negligible in a study by Brodowski et al. (2005). However, Glaser and Knorr (2008) showed by compound-specific stable-isotope analyses in condensed aromatic moieties similar to the ones of black carbon that significant amounts of black carbon in soils are formed by biological – or at least non-pyrogenic – processes in different soil types under different climatic conditions. The highest biological black carbon production of up to 9% annually relative to the total black carbon inventory was observed in a charcoal and manure-amended soil in Central Amazonia (Glaser and Knorr, 2008).

Concluding, there are indications for microbial processes in *terra preta* which promote sustainability. But these microbial processes in *terra preta* are similar to those also observed in “normal” soils and consist mainly of aerobic processes similar to those that occur during composting (Fig. 2.11). There is no evidence for anaerobic processes such as fermentation, which has been postulated recently (Factura et al., 2010). Furthermore, is it even more unlikely that fermentation was done in ceramic pots although potsherds are plentifully found in *terra preta* because it is most unlikely that thousands of cubic metres of *terra preta* is produced without mechanisation under these conditions.

In addition, it is clear that *terra preta* cannot regenerate itself when exploited as often done in Central Amazonia for economic reasons (soil mining). Although enhanced input of plant-derived nutrients such as C and N still can continue and maintain SOM levels, nutrient levels of other elements such as Ca, K, Mg, and P cannot regenerate without further addition of materials discussed in Section 2.5.3. In addition, from a scientific point of view, a soil's characteristics might persist for a long time in a state of equilibrium with the local environment. But it is not "regenerating" itself.

2.5.5 Intentional vs. unintentional terra preta creation

Terra preta is preferentially used for agriculture today by *Indios* (Schmidt and Heckenberger, 2009), *caboclos*, and modern farmers. Phytolith data has shown that this was the case already in pre-Columbian times (Bozarth et al., 2009). Our data presented and discussed above support the hypothesis that *terra preta* developed from midden areas with various inputs of organics (including mammal and fish bones, excrement and biochar) and inorganic material (including ash) and further debris (Fig. 2.11). It is possible that agricultural practices in home gardens contributed to the genesis of *terra preta* as well. Today, midden areas are used as home gardens or home gardens are used as trash areas by indigenous groups (Hecht, 2003; Schmidt and Heckenberger, 2009). Amendments of biochar to home gardens (see Section 2.5.2 Sources of biochar) are responsible for the high amounts of black carbon. Therefore, *terra preta* genesis can be explained by formation from midden areas and probably home garden agriculture as also practised today. Thus *terra preta* formation is likely a combination of both unintentional soil modification as well as intentional amendments to improve small-scale home gardens.

Pre-Columbian villages were more or less permanently settled or re-settled frequently due to easier clearance of secondary forest with stone axes and due to the enhanced soil fertility. These two facts could have boosted the genesis of well developed and large *terra preta* sites like a self-perpetuating or self-organization process (Denevan, 1998). In addition, there is no scientific evidence indicating that forgotten agricultural techniques for large scale soil fertility improvement are responsible for *terra preta* genesis.

2.6 Conclusions

The existence of *terra preta* even several thousand years after their creation unambiguously shows that improvement of highly weathered tropical soils by human actions is possible. More important, *terra preta* is a model for sustainable soil fertility and long-term C sequestration even today created by an intelligent material flow management. From scientific data discussed in this review it is very likely that *terra preta* was not intentionally created to improve soil fertility at large scales, at least not at the beginning. All available scientific data fit a genesis from midden eventually combined with home garden agriculture. It is most likely that (unintentional) creation of a fertile soil (*terra preta*) attracted more and more people and/or favoured population growth managing more and more resources (biochar, wastes, excrement) which set in motion selfenhancing and self-organizing processes.

Chapter 3

Faeces deposition on Amazonian Anthrosols as assessed from 5β-stanols

Birk J.J., Teixeira W.G., Neves E.G., and Glaser B., 2011.
Journal of Archaeological Science 38, 1209-1220.

3.1 Abstract

In the Amazon Basin, within a landscape of infertile soils, fertile Anthrosols of pre-Columbian origin occur (Amazonian Dark Earths or *terra preta de Índio*). These soils are characterized by high amounts of charred organic matter (black carbon, biochar) and high nutrient stocks. Frequently, they were considered as sign for intensive landscape domestication by way of sedentary agriculture and as sign for large settlements in pre-Columbian Amazonia. Beyond the archaeological interest in Amazonian Dark Earths, they increasingly receive attention because it is assumed that they could serve as a model for sustainable agriculture in the humid tropics (*terra preta nova*). Both questions lack information about the pre-Columbian practices which were responsible for the genesis of Amazonian Dark Earths. It has often been hypothesized that deposition of faeces could have contributed to the high nutrient stocks in these soils, but no study has focussed on this question yet. We analysed the biomarkers for faeces 5β-stanols as well as their precursors and their 5α-isomers in Amazonian Dark Earths and reference soils to investigate the input of faeces into Amazonian Dark Earths. Using Amazonian Dark Earths as example, we discuss the application of threshold values for specific stanols to evaluate faeces deposition in archaeological soils and demonstrate an alternative approach which is based on a comparison of the concentration patterns of 5β-stanols with the concentration patterns of their precursors and their 5α-isomers as well as with local backgrounds. The concentration patterns of sterols show that faeces were deposited on Amazonian Dark Earths.

3.2 Introduction

3.2.1 Amazonian Dark Earths and agriculture in Amazonia

Population sizes and extent of landscape domestication in pre-Columbian Amazonia are currently debated (Balée and Erickson, 2006; Erickson, 2008; Heckenberger et al., 2003, 2008; Heckenberger and Neves, 2009; McKey et al,. 2010; Meggers, 2003). For many years it has been assumed that infertile upland soils would have inhibited high population density and sedentary agriculture in the past (Meggers, 1954). Unanticipated from this point of view, patches of dark coloured and highly fertile Anthrosols of pre-Columbian origin were found in the Amazon Basin which are known as Amazonian Dark

Earths (ADE) or *terra preta de índio* (e.g. Glaser, 2007; Glaser et al., 2004a; Lehmann et al., 2003b; Petersen et al., 2001; Sombroek, 1966; Woods et al., 2009).

The available numeric data for the beginning of ADE formation generally range between the middle of the first millennium BC and the first millennium AD and they were correlated to the appearance of the Incised Rim tradition and the Polychrome tradition (Neves, 2008; Petersen et al., 2001).

Up to now, the socio-cultural or economic practices generating ADE in the past are not yet understood (Glaser, 2007; Lehmann, 2009). Despite the limited knowledge about their genesis, the occurrence of ADE was considered as sign for sedentary agriculture and large settlements in the Amazon Basin during pre-Columbian times (Denevan, 1996; Heckenberger and Neves, 2009; Neves et al., 2003; Petersen et al., 2001; Smith, 1980).

Beyond the archaeological interest in ADE, these soils receive increasing interest because of their persisting fertility showing that marginal upland soils in the humid tropics can be improved sustainably. In this way, ADE could serve as a model for sustainable agriculture in the humid tropics (Glaser, 2007; Heckenberger and Neves, 2009; Lehmann, 2009; Sombroek et al., 2003; Woods and McCann, 1999).

3.2.2 Geochemical properties and genesis of ADE

Both the study of pre-Columbian landscape domestication and the development of new agricultural techniques for the humid tropics lack information about the pre-Columbian practices which were responsible for the ADE formation. The main geochemical features which distinguish these soils from surrounding Ferralsols, Acrisols and Arenosols are a constantly high cation exchange capacity and high nutrient levels (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b, 2003; Kämpf and Kern, 2005; Lehmann et al., 2003a; Sombroek, 1966; Zech et al., 1990). The high cation exchange capacity is maintained by a stable pool of soil organic matter (SOM) (Glaser, 2007; Glaser et al., 2001a, 2001b; Zech et al., 1990). Glaser et al. (2001a) identified charred organic matter (black carbon; biochar) as a key factor for the stability of the SOM in these soils. Also, microbially derived stable SOM is assumed to contribute to the stable SOM pool (Glaser, 2007).

The second factor, which causes the fertility of ADE, is their high nutrient stock. First of all they are enriched in plant-available P (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b; Lehmann et al., 2003a; Smith, 1980; Sombroek, 1966; Zech et al., 1990). Also total P is several times higher in ADE than in surrounding soils

(Costa and Kern, 1999; Lehmann et al., 2003a). Plant-available and total Ca, N and Mg are strongly enriched in relation to surrounding soils and amounts of K, Zn and Mn were also found at higher levels in ADE (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b; Lehmann et al., 2003a; Sombroek, 1966; Zech et al., 1990).

Only a few studies of the nutrient sources have been carried out so far (Glaser, 2007). Ash may have been a significant input into ADE (Arroyo-Kalin et al., 2009; McCann et al., 2001; Woods, 2003b). Waste, including mammal and fish bones likely contributes to the nutrient stocks. For example Lima et al. (2002) and Schaefer et al. (2004) found evidence for high Ca and P contents linked to bones in ADE.

This study focuses on faecal material as possible nutrient source. It has often been hypothesized that faeces had been a nutrient source in ADE (Glaser, 2007; Glaser et al., 2004a; Smith, 1980; Sombroek, 1966; Woods, 2003b), but no study has focussed on this question yet.

3.2.3 5β-stanols as biomarkers for faecal input into archaeological soils

The potential of 5β-stanols as biomarkers to detect faecal input to archaeological soils and sediments has already been extensively investigated (Bethel et al., 1994; Bull et al., 1998, 1999b, 2002, 2005; Evershed, 2008; Evershed and Bethell, 1996; Evershed et al., 1997; Hjulström and Isaksson, 2009; Knights et al., 1983; Simpson et al., 1999b). 5ß-stanols originate mainly from microbial reduction of Δ^5-sterols in the gut of mammals. 5β-cholestan-3β-ol is the most abundant stanol in the faeces of omnivores, while high amounts of 5β-stigmastan-3β-ol occur in the faeces of herbivores. Using this difference, 5β-stanol patterns can be used to gain information about the origin of faeces in environmental samples (Bethel et al., 1994; Evershed and Bethell, 1996; Gill et al., 2010; Leeming et al., 1997).

However, 5β-stanols were also found in soils where no anthropogenic deposition of faeces was assumed, showing that the presence of 5β-stanols alone is not sufficient to proof ancient faecal deposition on soils (Bethel et al., 1994; Bull et al., 2001; Evershed et al., 1997). Additionally, elevated total concentrations of 5β-stanols in soils could also be caused by enhanced concentrations of their precursors (Δ^5-sterols), which were reduced in situ and by an enhanced stabilization. However, the majority of Δ^5-sterols are transformed to 5α-stanols by way of reduction in the environment and only a minor amount is reduced to 5β-stanols (Fig. 3.1). To evaluate anthropogenic input of faeces into soils independently from total concentrations, the amounts of 5β-stanols are related to the amounts of 5α-stanols in soil samples [e.g. 5β-cholestan-3β-ol/(5α-cholestan-3β-ol + 5β-cholestan-3β-ol)] (Bull et al., 1999a, 1999b, 2002, 2005; Simpson et al., 1998) as proposed by Grimalt et al. (1990) for water particulates and sediment samples. In order to correct for microbial epimerisation of 5β-cholestan-3β-ol to 5β-cholestan-3α-ol in soils, this epimer was induced in the 5β/(5β + 5α)-cholestanol ratio [(5β-cholestan-3β-ol + 5β-cholestan-3α-ol) /(5α-cholestan-3β-ol + 5β-cholestan-3β-ol + 5β-cholestan-3α-ol)] (Bull et al., 1999a, 1999b, 2002, 2005; Simpson et al., 1998).

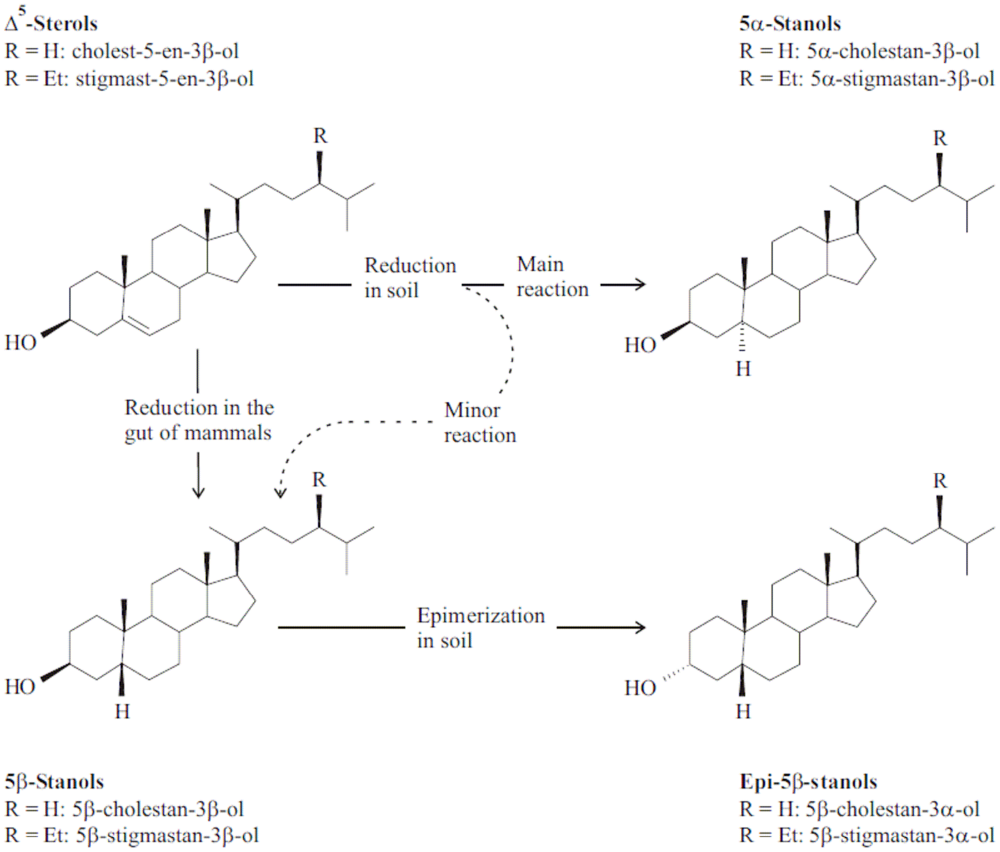

Figure 3.1 Products of Δ^5-sterol reduction in the gut of mammals and in soils (Bull et al., 1999b, 2002).

Grimalt et al. (1990) found that samples from coastal areas without and with anthropogenic input of faeces showed 5β/(5β + 5α)-cholestanol ratios <0.3 and ≥0.7, respectively. These threshold values were cited in further studies (e.g. Bull et al., 1999a, 2001, 2003; Elhmmali et al., 2000; Fitzsimons et al., 1995; Jeng and Han, 1994; Simpson et al., 1998). However, the application of these threshold values in archaeological samples was critically discussed e.g. by Bull et al. (1999a, 1999b, 2001, 2005) and Simpson et al. (1998) and a comparison with local background samples was recommended instead by Bull et al. (1999b).

In this study, we followed an alternative approach based on a comparison of the 5β-stanol concentrations and 5β /(5β + 5α)-stanol ratios (i) with the concentration patterns of the precursors of the 5β-stanols and their 5α-isomers and (ii) with local backgrounds in reference soils.

3.3 Materials and methods

3.3.1 Sites and sampling

Five typical ADE and adjacent reference soils were analysed. ADE occur in whole Amazonia at places with varying ecological characteristics and their formation was associated with different pre-Columbian cultures (Denevan, 1996; Glaser, 2007; Kern et al., 2003; McCann et al., 2001; Neves, 2008; Neves et al., 2004; Petersen et al., 2001; Rebellato et al., 2009; Smith, 1980; Sombroek, 1966). Therefore, the properties of ADE differ to some extent between sites as well as within sites and the formation process was likely not completely identical at all sites (Kern et al., 2003; Neves et al., 2003). However, all typical ADE show the same characteristic geochemical differences from surrounding soils (Section 3.2.2). This indicates that the main processes involved in their formation were comparable. The aim of this study was to investigate if the deposition of faeces was a general source for the nutrients in ADE regardless of the specific culture which settled on a site and the specific ecological conditions. Therefore, we choose a range of typical ADE instead of sites of a single culture or sites at places with distinct ecological characteristics. For this purpose, four ADE were sampled in the southwest of Manaus on the headland between the Rio Solimões and the Rio Negro (Hatahara, Açutuba, and two sites in the area of the Caldeirão Embrapa research station) and one ADE was sampled in the southwest of Santarém at the lower Tapajós (Belterra).

All sampled ADE had negligible slopes and were located in non-floodable positions (*terra firme*) close to the floodplains of rivers. These areas are the typical positions where ADE has been found (Kern et al., 2003). Since ADE developed mostly from Ferralsols and Acrisols (Kern et al., 2003), we selected sites associated to these soil types. ADE were identified by their thick black A horizons which were 40 – 80 cm thick and contained ceramics and charcoal particles in a high density (Kämpf et al., 2003). Below the A horizons followed transition horizons to the B horizons which were several decimetre thick and showed patches of different brown, grey and black colours. The B horizons of the ADE were identical to the B horizons of Ferralsols and Acrisols. For sampling, first the center of the ADE was identified by thoroughly augering of the whole site including the surrounding soils.

The ADE sampled in the southwest of Manaus were located close to the Rio Negro (black water) or close to the Rio Solimões (white water). The textures of these soils were loamy. In the area around the confluent of the Rio Negro and the Rio Solimões the formation of ADE began with the Manacaparu phase (Barrancoid or Incised Rim tradition) and continued during Paredão and Guarita occupations (Amazon Polychrome tradition) (Neves et al., 2004; Petersen et al., 2001; Rebellato et al., 2009). The ADE sampled in the southwest of Santarém was located close to the Rio Tapajós (clear water) and the texture of this site was clayey. In the Santarém area large extensions of ADE were found associated with Incised Punctuated sites which can overlap older occupations from the Pocó phase (Neves, 2008; Roosevelt, 1999).

Within a distance <1 km to each ADE a reference soil was sampled. Our main criteria to identify non-ADE sites were the absence of ceramics, the thickness of the A horizon (≈10 cm) and a lighter more brownish colour of the A horizon which was typical for Ferralsols and Acrisols. The AB horizons showed continuous transition from the colours of the A horizons to the yellowish brownish or reddish brownish colours of the B horizons. All sampled reference sites were located in the same topographical position like the associated ADE and they had a texture which was similar to the texture of the ADE site in the topsoil and subsoil, respectively. The similar textures of ADE sites and the surrounding soils indicated that they developed on the same parent material and that the A horizons of the ADE were not build by transportation of soil material from elsewhere e.g. floodplains (Glaser et al., 2004a).

The 0 – 10 cm and 30 – 40 cm soil depths were chosen for steroid analysis. The depth 30 – 40 cm was within the middle or lower part of the A horizons of all ADE and within the B horizon of the reference soils. It was assumed that this soil depth was less influenced by recent land use and recent vegetation cover than the shallower soil above. The soil depths 0 – 10 cm covered in most cases the complete A horizon of the reference soils. It allowed comparison of the data from both depths of the ADE with the A horizons of the reference soils. Furthermore, a comparison of the samples from the depth 30 – 40 cm and the soil close to the surface in ADE as well as in reference soils was possible due to this sampling design.

Table 1 summarizes the basic soil parameters of the investigated samples. The samples from the reference soils had low pH values, low effective cation exchange capacities and low amounts of organic carbon, exchangeable basic cations and plant-available phosphor which are characteristics for Ferralsols and Acrisols (IUSS Working Group WRB, 2006; Sombroek, 1966). In contrast the ADE showed the typical enhancement of these variables in both soil depths (Costa and Kern, 1999; Falcão et al., 2009; Glaser, 2007; Glaser et al., 2001b; Lehmann et al., 2003a; Smith, 1980; Sombroek, 1966; Zech et al., 1990).

Table 3.1 Basic characteristics of soil samples from 0 to 10 and from 30 to 40 cm depths in soils from the Brazilian Amazon region [data from Glaser (1999) and unpublished data].

	Depth (cm)	pH[a] (KCl)	TOC[b] ($g\ kg^{-1}$)	N_{total}[c] ($g\ kg^{-1}$)	CEC_{eff}[d] ($mmol_c\ kg^{-1}$)	BS[e] (%)	P_{NaHCO3}[f] ($mg\ kg^{-1}$)
Amazonian	0 – 10	5.8±0.4	45.1±11.8	3.2±0.8	152±47	98±1	95±26
Dark Earth	30 – 40	4.9±0.3	20.3±5.9	1.1±0.3	107±62	95±3	191±38
Reference	0 – 10	3.8±0.1	15.3±1.5	1.1±0.1	61±46	6±3	4±1
	30 – 40	4.1±0.1	7.7±1.8	0.6±0.2	33±21	3±1	2±0

[a] Data from four profile pairs;
[b] Total organic carbon;
[c] Total nitrogen;
[d] Cation exchange capacity (effective), data from four profile pairs;
[e] Base saturation, data from three profile pairs;
[f] Extracted with $NaHCO_3$, data from four profile pairs.

3.3.2 Analytical procedures

Steroids were extracted and purified according to the method described by Isobe et al. (2002), with small modifications. Briefly, 5 g air-dried and finely ground soil were spiked with 5α-pregnan-3β-ol as recovery standard and ultrasonically extracted with MeOH, MeOH/dichloromethane (DCM) (1:1, v/v) and DCM consecutively. For saponification, the dried extracts were heated (80 °C; 3 h) with 5% KOH/MeOH. The sterols were extracted from the KOH/MeOH solution by repeated solvent extraction with hexane.

5% H_2O-deactivated silica gel columns (100 – 200 mesh) were used for purification. Subsequently, three fractions were eluted. The first fraction was eluted with hexane/DCM (3:1, v/v) and discarded. The sterols were eluted from the columns with DCM (second fraction) and acetone/DCM (3:7, v/v; third fraction). The second and the third fraction were collected and combined to the sterol fraction.

Derivatization was carried out by heating (70 °C; 1 h) the dried sterol fraction in *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA), containing 1% trimethylchlorosilane (TMCS) (Bull et al., 1999a; Simpson et al., 1998; Van Bergen et al., 1998).

α-Cholestane was added as a second internal standard and the sterols were analysed using gas chromatography-mass spectrometry (GC/MS) in selected ion monitoring (SIM) mode with a Hewlett-Packard 5973 quadrupole mass spectrometer fitted with an HP 6890 gas chromatograph. A HP-5ms, 30 m fused silica column with 0.25 mm i.d. and 0.25 µm film thickness was used for sterol separation. He (99.9990% purity) was used as carrier gas at 86 kPa constant pressure. The injection port was set to 270 °C and the sample was injected in splitless mode. The column temperature programme was: 80 °C (0.5 min) to 235 °C at 20 °C/min and to 300 °C (held 12 min) at 2 °C/min.

3.3.3 Calculations

The peak areas of the sterols in the samples and in the external standards, respectively, with the exception of 5β-stigmastan-3β-ol, were divided by the peak area of the second internal standard. Quantification of these sterols was achieved from calibration curves ($R^2 > 0.98$) drawn for individual sterols using standard solutions containing 5α-cholestane, 5α-pregnan-3β-ol, cholest-5-en-3β-ol, 5β-cholestan-3β-ol, 5β-cholestan-3α-ol, 5α-cholestan-3β-ol, 24-ethyl-cholest-5-en-3β-ol, (stigmast-5-en-3β-ol) and 24-ethyl-5α-cholestan-3β-ol (5α-stigmastan-3β-ol). 24-ethyl-5β-cholestan-3β-ol (5β-stigmastan-3β-ol) was identified from ion chromatograms measured in SCAN mode and quantified relative to the first standard by dividing the area from 5β-stigmastan-3β-ol in the *m/z* 215 SIM chromatogram by the area from 5α-pregnan-3β-ol in the *m/z* 361 SIM chromatogram (i.e. relative abundances). Using the same method for extraction and purification, Isobe et al. (2002) found coefficients of variation between 4 and 13% for a sample of cow faeces. Recoveries of the first internal standard >80% for each sample in our study also indicated a reproducibility and precision of the method better than ±20%

without correction of losses during extraction and purification. We corrected for such losses by multiplying the amounts of each analyte with the reciprocal value of the recovery (values in percent were divided by 100).

Whenever the amounts of 5β-cholestan-3β-ol and 5β-cholestan-3α-ol in the reference soils were below detection limit or below the range of the external calibration curves, the lowest amounts which were within the range of the external calibration curves were used as the actual substance amounts, respectively (approximately 0.8 ng g_{soil}^{-1}; depending on the exact weight of soil used for the analysis). This was done to avoid overestimation of the difference between ADE and the reference soils with respect to these two substances.

Normality of paired differences for each location between ADE and the adjacent soil was tested using Kolmogornov-Smirnov-test. For variables that failed the normality tests, the natural logarithm was calculated to transform the data and to meet normality. In order to test for significance of differences between ADE and reference soils, paired t-tests were calculated. To test for relationships between variables, Spearman's rank correlation coefficients were calculated.

3.4 Results

3.4.1 Amounts of Δ^5-sterols and non-faecal derived stanols

Stigmast-5-en-3β-ol and its reduction product 5α-stigmastan-3β-ol were found in higher concentrations than cholest-5-en-3β-ol and its reduction product 5α-cholestan-3β-ol, respectively (Fig. 3.2 and Fig. 3.3). In the upper 10 cm of ADE, all of these sterols had significantly elevated amounts compared to the reference soils. At 30 – 40 cm soil depth, the concentrations were considerably lower than at 0 – 10 cm soil depth and only minor differences in the concentrations of these sterols were found between ADE and reference soils. For both ADE and references, concentrations of the deeper samples showed a stronger decrease for stigmast-5-en-3β-ol and 5α-stigmastan-3β-ol than for cholest-5-en-3β-ol and 5α-cholestan-3β-ol. The similarity between the concentration patterns of 5α-stanols and their precursors (cholest-5-en-3β-ol and stigmast-5-en-3β-ol, respectively) was underlined by distinct correlations between these substances (Fig. 3.4).

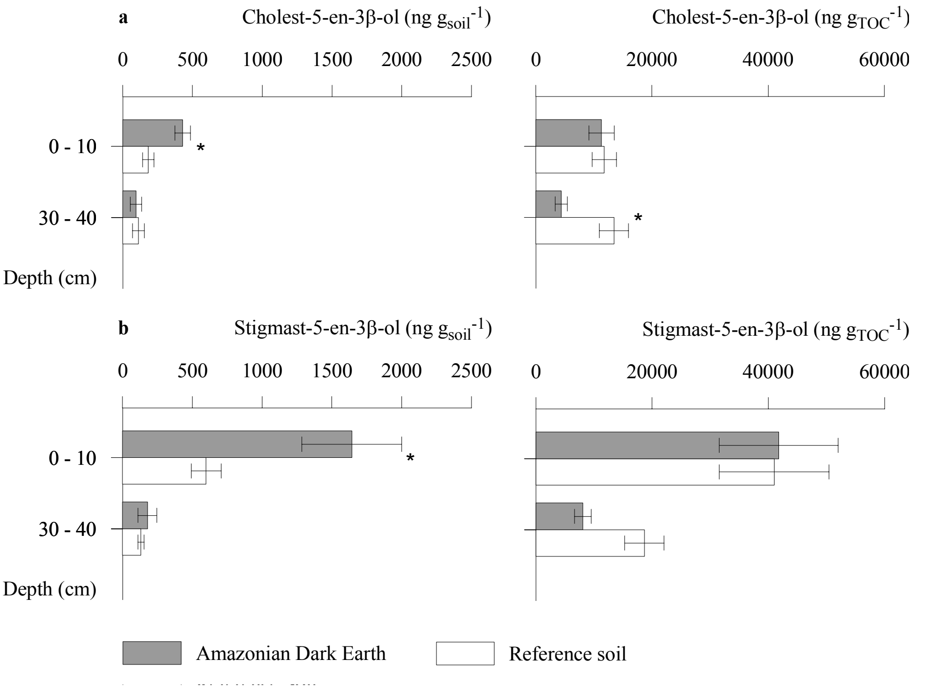

Figure 3.2 Concentrations of Δ^5-sterols (a) cholest-5-en-3β-ol and (b) stigmast-5-en-3β-ol. Means of amounts in bulk soil (left) and relative to TOC (right) in ADE and reference soils [* indicates significant differences ($P < 0.05$) between ADE and reference soils; error bars show standard errors; $n = 5$].

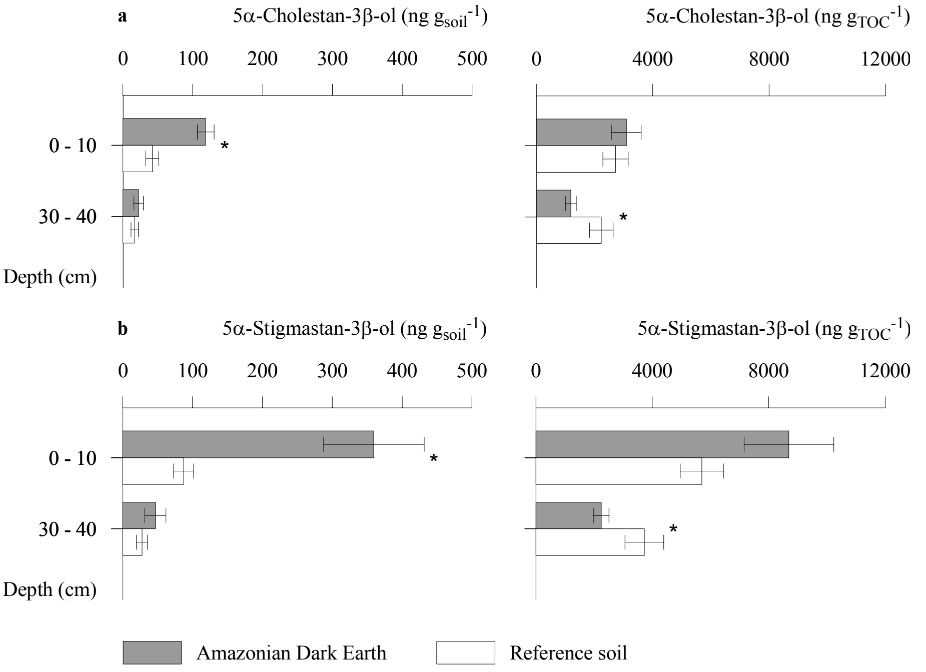

Figure 3.3 Concentrations of 5α-stanols (a) 5α-cholestan-3β-ol and (b) 5α-stigmastan-3β-ol. Means of amounts in bulk soil (left) and relative to TOC (right) in ADE and reference soils [* indicates significant differences ($P < 0.05$) between ADE and reference soils; error bars show standard errors; $n = 5$].

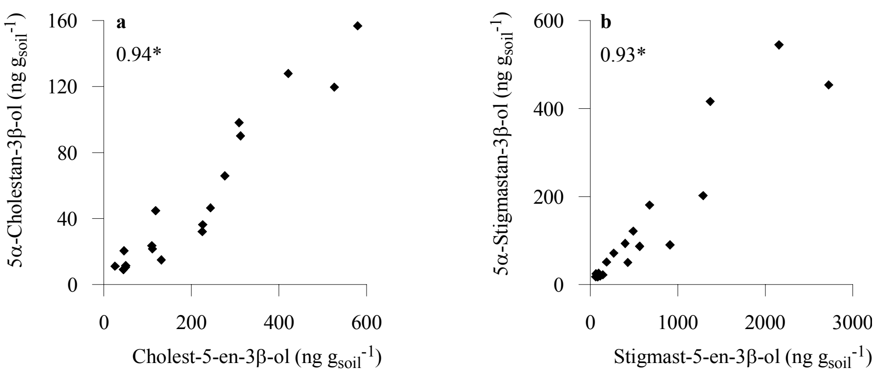

Figure 3.4 Amounts of Δ^5-sterols vs. amounts of their characteristic reduction products in soils (a) 5α-cholestan-3β-ol and (b) 5α-stigmastan-3β-ol in ADE and reference soils. Numbers show correlation coefficients (Spearman) and * indicates significant correlations ($P < 0.05$).

The amounts of Δ^5-sterols and 5α-stanols relative to total organic carbon (TOC) did not show significant differences in the upper 10 cm (Fig. 3.2 and Fig. 3.3). In ADE, the concentrations relative to TOC of all these components were considerably lower at 30 – 40 cm soil depth than in the upper 10 cm. In the reference soils, the amounts of stigmast-5-en-3β-ol and 5α-stigmastan-3β-ol relative to TOC were also reduced in relation to the upper 10 cm but to a lower extent than in the ADE. The concentrations relative to TOC of cholest-5-en-3β-ol and 5α-cholestan-3β-ol differed only slightly between both depths in the reference soils. Thus, the amounts of Δ^5-sterols and 5α-stanols relative to TOC were enhanced in the reference soils in relation to ADE at 30 – 40 cm soil depth.

3.4.2 Amounts of the faecal biomarkers 5β-cholestan-3β-ol and 5β-cholestan-3α-ol

The concentrations of 5β-cholestan-3β-ol did not differ significantly between ADE and references in the upper 10 cm (Fig. 3.5a). However, the amounts of its epimer 5β-cholestan-3α-ol, were elevated in ADE at 0 – 10 cm soil depth in relation to the reference soils (Fig. 3.5b; at 0 – 10 cm soil depths one ADE had extremely high 5β-cholestan-3α-ol amounts of 24.9 ng g_{soil}^{-1}. This outlier and all further 5β-cholestan-3α-ol data of this ADE and the corresponding reference were excluded from all figures and statistical analyses). In the reference soils, the amounts of 5β-cholestan-3β-ol and 5β-cholestan-3α-ol decreased at 30 – 40 cm soil depth compared to the upper 10 cm. In ADE, the concentration of 5β-cholestan-3β-ol was similar at 0 – 10 cm and at 30 – 40 cm soil depth and the concentration of 5β-cholestan-3α-ol at 30 – 40 cm depth was decreased to a lower extent in relation to the upper 10 cm than the non-faecal derived sterols. Thus, their concentrations in ADE at 30 – 40 cm soil depth were higher than in the reference soils.

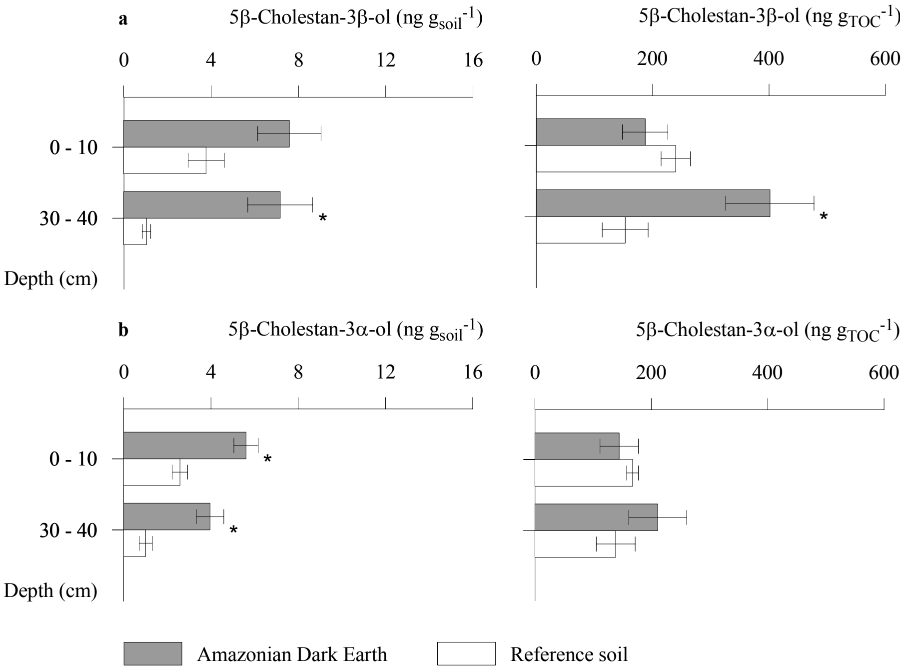

Figure 3.5 Concentrations of faecal biomarkers (a) 5β-cholestan-3β-ol and (b) 5β-cholestan-3α-ol. Means of amounts in bulk soil (left) and relative to TOC (right) in ADE and reference soils [* indicates significant differences ($P < 0.05$) between ADE and reference soils; error bars show standard errors; n = 5 for 5β-cholestan-3β-ol; n = 4 for 5β-cholestan-3α-ol.].

No significant correlations were found between the amounts of 5β-cholestan-3β-ol and the amounts of its isomer 5α-cholestan-3β-ol and the corresponding Δ^5-sterol cholest-5-en-3β-ol, respectively (Fig. 3.6a). The amounts of 5β-cholestan-3α-ol significantly correlated with the amounts of cholest-5-en-3β-ol and 5α-cholestan-3β-ol, respectively (Fig. 3.6b). A significant correlation was also found between the amounts of 5β-cholestan-3β-ol and the amounts of 5β-cholestan-3α-ol (Fig. 3.6c).

No significant differences between ADE and reference soils were found for the amounts of 5β-cholestan-3β-ol and 5β-cholestan-3α-ol relative to TOC in the upper 10 cm (Fig. 3.5). Also at 30 – 40 cm soil depth, the amounts of 5β-cholestan-3α-ol relative to TOC did not differ significantly between ADE and reference soils. However, the concentrations of 5β-cholestan-3β-ol relative to TOC were significantly higher in ADE at 30 – 40 cm soil depth than in the reference soils.

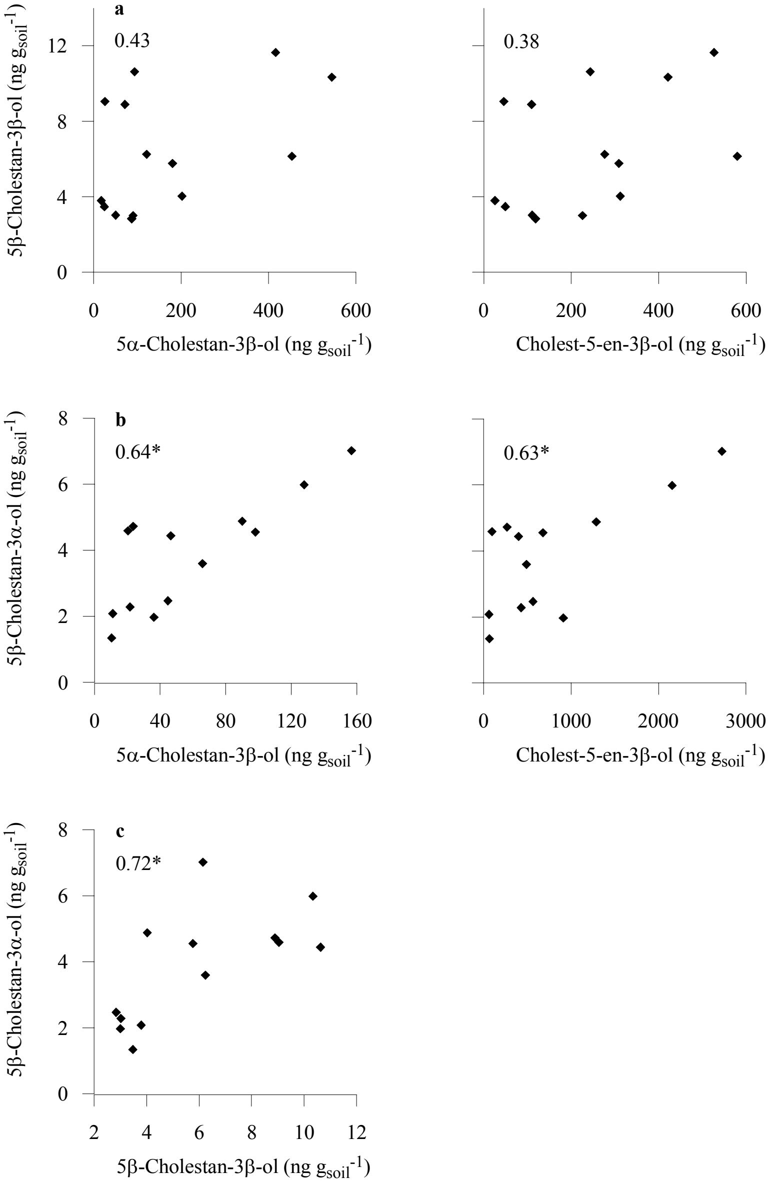

Figure 3.6 Amounts of faecal biomarkers (a) 5β-cholestan-3β-ol and (b) 5β-cholestan-3α-ol. vs. amounts of their 5α-isomer (left), amounts of correlated Δ^5-sterols (right) and (c) amounts of their epimers in ADE and reference soils. Numbers show correlation coefficients (Spearman) and * indicates significant correlations ($P < 0.05$).

3.4.3 Relative abundances of the faecal biomarker 5β-stigmastan-3β-ol

The relative abundances of the biomarker for faeces of herbivores, 5β-stigmastan-3β-ol were higher in the upper 10 cm of ADE than in reference soils (relative abundances in ADE: 0.279 ± 0.062; relative abundances in reference soils: 0.092 ± 0.032; data not shown). At 30 – 40 cm soil depth, 5β-Stigmastan-3β-ol was not frequently detectable and in any case at a lower relative abundance than in the upper 10 cm (relative abundances in two ADE samples: 0.059 and 0.095 and in one reference sample: 0.046; data not shown). The relative abundances of 5β-stigmastan-3β-ol correlated with that of its corresponding 5α-isomer (5α-stigmastan-3β-ol, Fig. 3.7a) and the corresponding Δ^5-sterol (stigmast-5-en-3β-ol, Fig. 3.7b).

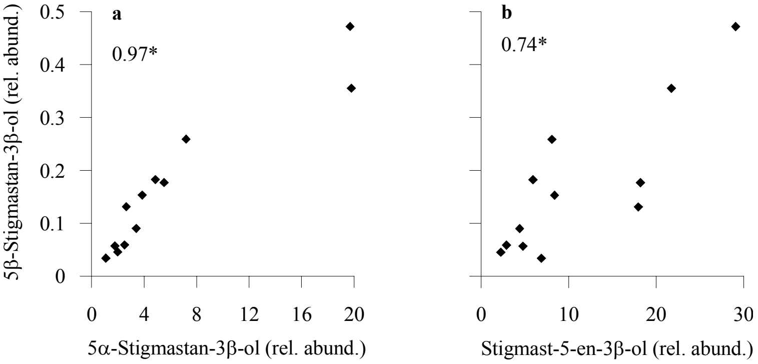

Figure 3.7 Amounts of the faecal biomarker 5β-stigmastan-3β-ol vs. (a) amounts of its 5α-isomer and (b) amounts of its correlated Δ^5-sterols in ADE and reference soils. Numbers show correlation coefficients (Spearman) and * indicates significant correlations ($P < 0.05$).

3.4.4 5β/(5β + 5α)-stanol ratios

The amounts of 5β-cholestan-3β-ol (faecal biomarker) were related to those of the 5α-cholestan-3β-ol (reduction in soils) as proposed by Grimalt et al. (1990) enabling the detection of enhanced input of faeces [5β-cholestan-3β-ol/(5α-cholestan-3β-ol + 5β-cholestan-3β-ol)]. In ADE as well as in the reference soils, this ratio was below 0.3. The ratio incorporating the epimer of 5β-cholestan-3β-ol [(5β-cholestan-3β-ol + 5β-cholestan-3α-ol)/(5α-cholestan-3β-ol + 5β-cholestan-3β-ol + 5β-cholestan-3α-ol)] (Bull et al., 1999a, 1999b, 2002; Simpson et al., 1998) reached a mean value between 0.3 and 0.7, but only in ADE at 30 – 40 cm soil depth.

Both ratios did not distinguish ADE from the reference soils in the upper 10 cm (Fig. 3.8). At 30 – 40 cm soil depth, the ratios were significantly higher in ADE than in the reference soils. In the reference, soils the ratios at 30 – 40 cm soil depth differed only slightly from the upper 10 cm. In contrast the ratios of the ADE were considerably higher at 30 – 40 cm soil depth in relation to the upper 10 cm. Thus, the ratios were also significantly higher at 30 – 40 cm soil depth of the ADE than in the shallowest 10 cm of the reference soils.

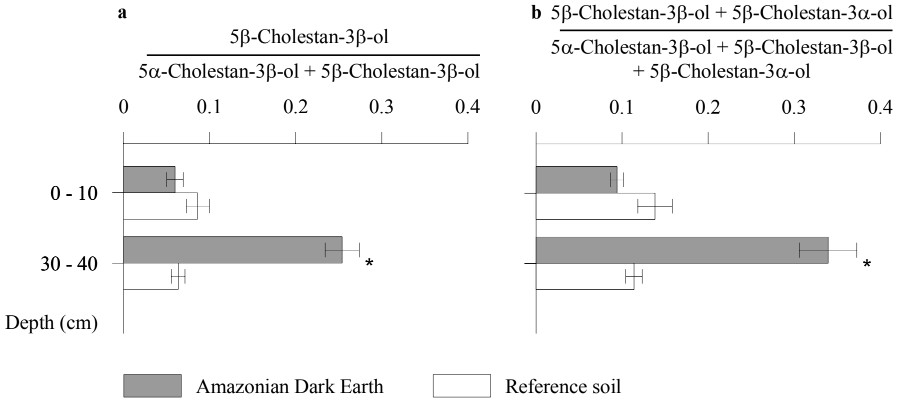

Figure 3.8 5β/(5β + 5α)-cholestanol ratios (a) according to Grimalt et al. (1990) and (b) including 5β-cholestan-3α-ol in ADE and reference soils. Higher values indicate greater deposition of faecal material [*indicate significant differences ($P < 0.05$) between ADE and reference soils; error bars show standard errors; $n = 5$ for a; $n = 4$ for b].

The ratio of the relative abundances of 5β-stigmastan-3β-ol/(5α-stigmastan-3β-ol + 5β-stigmastan-3β-ol) was 0.031 ± 0.005 for the upper 10 cm of ADE (data not shown). The reference soils exhibited a ratio of 0.033 ± 0.002 in the upper 10 cm (data not shown). Thus, the ratios were very similar between ADE and reference soils in this depth. At 30 – 40 cm soil depth, 5β-stigmastan-3β-ol was only detected in two ADE samples and the ratios in these samples were lower than in the upper 10 cm (0.023 and 0.026; data not shown). 5β-stigmastan-3β-ol was only found in one reference soil at 30 – 40 cm soil depth, whereas the ratio was 0.022 in this sample (data not shown).

3.5 Discussion

3.5.1 Threshold values to detect ancient faecal deposition on ADE vs. comparison to reference soils

In the ADE samples, the 5β/(5β + 5α)-cholestan-3β-ol ratios were lower than the upper value of 0.3 which was found by Grimalt et al. (1990) for samples without anthropogenic input of faeces. Consequently, anthropogenic faecal input into these soils should be excluded. However, the modified ratio [(5β-cholestan-3β-ol + 5β-cholestan-3α-ol)/(5β-cholestan-3β-ol + 5β-cholestan-3α-ol + 5α-cholestan-3β-ol)] at 30 – 40 cm soil depth of ADE was higher than the threshold value of 0.3, but still lower than 0.7. According to the values of Grimalt et al. (1990), it would be neither possible to proof nor to exclude anthropogenic faecal input into ADE .

Grimalt et al. (1990) analyzed water particulates and sediments sampled in the coastal environments of Spain and the Cuba Bay. Only in samples derived from sewage-polluted areas in Spain, these authors found 5β /(5β + 5α)-cholestan-3β-ol ratios ≥0.7. In sediment samples originating from the heavily polluted Cuba Bay, this threshold value was not reached but the ratio was above 0.3. At the same time this upper value for the exclusion of contamination was exceeded in some samples from uncontaminated areas. Additionally, Isobe et al. (2002) showed that the threshold value of 0.7 can not be applied in general. In terrestrial soils of Central Amazonia, the threshold value could fail as well as in the heavily polluted Cuba Bay investigated by Grimalt et al. (1990). These authors identified the high input of 5α-cholestan-3β-ol by algae as reason for 5β /(5β + 5α)-cholestan-3β-ol ratios <0.7 in the heavily polluted Cuba Bay. In Central Amazonia, comparable effects could cause 5β /(5β + 5α)-cholestan-3β-ol ratios <0.7, despite ancient faecal deposition. Forests in Amazonia belong to the terrestrial ecosystems with the highest plant productivity, as long as soils are not too heavily degraded. Therefore, litter-derived Δ^5-sterols can be a significant source for sterols and their reduction products in these soils, and thus 5α-stanols must be considered to have a high impact on the sterol patterns. Additionally, high decomposition rates of SOM characterize the soils in the humid tropics and Bull et al. (1999a, 1999b, 2001, 2005) and Simpson et al. (1998) generally expected a decrease of 5β /(5β + 5α)-cholestanol ratios in archaeological soils over time due to a lower thermodynamic stability of 5β-stanols in relation to 5α-stanols (Mackenzie et al., 1982). Thus, after the abandonment of ADE by pre-Columbian inhabitants both could have caused a decrease of the 5β /(5β + 5α)-cholestanol ratios: (i)

Decomposition of anciently deposited stanols including faeces derived 5β-cholestan-3β-ol combined with a continuous dilution of anciently deposited stanols by litter fall and (ii) a decrease in the 5β /(5β + 5α)-cholestanol ratios over time due to the lower thermodynamic stability of 5β-stanols in relation to 5α-stanols. Therefore, in ADE and other soils, where comparable effects could have caused a decrease of the 5β /(5β + 5α)-cholestanol ratios, it is problematic to use threshold values which were defined and applied for recent faeces inputs in different ecosystems, especially sediments by Grimalt et al. (1990). In our opinion, it is more appropriate to evaluate the validity of 5β-stanols as biomarkers for faeces in archaeological soils by comparing the 5β-stanol concentrations and 5β /(5β + 5α)-stanol ratios with the concentration patterns of the precursors of the 5β-stanols and their 5α-isomers as well as with local backgrounds in reference soils.

3.5.2 Concentrations of Δ^5-sterols and non-faecal derived stanols

In contrast to elevated absolute amounts of Δ^5-sterols and 5α-stanols in the upper 10 cm of the ADE compared to the reference soils, differences in the amounts of Δ^5-sterols and 5α-stanols related to TOC were not apparent. Thus, the differences of the absolute amounts in this depth could be explained by elevated SOM contents in ADE vs. reference soils. In addition, the difference between the upper 10 cm and the 30 – 40 cm soil depths was less pronounced for the concentrations relative to TOC than for absolute amounts of Δ^5-sterols and 5α-stanols, respectively. The remaining decrease in the concentrations of all Δ^5-sterols and 5α-stanols relative to TOC in ADE and stigmast-5-en-3β-ol and 5α-stigmastan-3β-ol in the reference soils at 30 – 40 cm soil depth in relation to the upper 10 cm could be caused by an enrichment of more stable or stabilized SOM in relation to sterols and labile SOM in deeper soil. This relative enrichment of stable SOM seemed to be more pronounced in the black carbon-rich ADE than in the reference soils, which could explain the lower concentration of Δ^5-sterols and 5α-stanols in the SOM of the 30 – 40 cm soil depth of the ADE vs. the reference soils. The more pronounced decrease in stigmast-5-en-3β-ol and 5α-stigmastan-3β-ol vs. cholest-5-en-3β-ol and 5α-cholestan-3β-ol could be caused by different sources for stigmast-5-en-3β-ol vs. cholest-5-en-3β-ol. Stigmast-5-en-3β-ol originates from plant tissue (Ibanez et al., 2000; Puglisi et al., 2003) and seem to be introduced into soils mainly at the surface or in shallow horizons by plant litter and roots. Cholest-5-en-3β-ol also occur in plant tissue but eucaryiotic soil organisms form an additional source (Ibanez et al., 2000; Puglisi et al., 2003), which could cause slightly more homogenous distribution in the soil profile.

3.5.3 Concentrations of biomarkers characteristic for faeces (5β-stanols) and 5β/(5β + 5α)-stanol ratios

The very similar 5β/(5β + 5α)-cholestan-3β-ol ratios for both depths in the reference soils and in the upper 10 cm of ADE could be considered as hints that 5β-cholestan-3β-ol originated from in situ Δ^5-sterol reduction in soil, like 5α-stanols do. However, the relatively high levels of the absolute amounts of 5β-cholestan-3β-ol and of its concentrations relative to TOC at the 30 – 40 cm soil depth of the ADE in relation to the reference soils did not fit this interpretation. Likewise, the higher 5β/(5β + 5α)-cholestan-3β-ol ratios in this depth of the ADE vs. the reference soils indicated that the 5β-cholestan-3β-ol and the 5α-cholestan-3β-ol in ADE originated from different sources. As 5α-cholestan-3β-ol is the product of cholest-5-en-3β-ol reduction in soil, this indicated that the 5β-cholestan-3β-ol in ADE did not mainly originate from in situ reduction of cholest-5-en-3β-ol.

Preferential formation or stabilization of 5β-cholestan-3β-ol originating from reduction of cholest-5-en-3β-ol in soil seems to be an unlikely interpretation. This would implicate strong differences in the formation or stabilization of 5β-cholestan-3β-ol in comparison to the formation or stabilization of 5α-stanols, which were not elevated at 30 – 40 cm soil depth in ADE to the same extent. So this interpretation would be contradictory to the use of 5β/(5β + 5α)-stanol ratios, which had been shown to be an indicator for faecal deposition.

Furthermore, assuming cholest-5-en-3β-ol reduction in soil as a source for 5β-cholestanl-3β-ol would also implicate strong differences in the accumulation of 5β-cholestan-3β-ol in comparison to the accumulation of 5β-stigmastan-3β-ol. In contrast to 5β-cholestan-3β-ol, 5β-stigmastan-3β-ol only occurred sporadically at 30 – 40 cm soil depth in ADE, despite the concentrations of stigmast-5-en-3β-ol and 5α-stigmastan-3β-ol were higher than the concentrations of cholest-5-en-3β-ol and 5α-cholestan-3β-ol, respectively. Thus, assuming a preferential accumulation of in situ cholest-5-en-3β-ol-derived 5β-cholestan-3β-ol would generally challenge the use of the ratio of the faecal biomarkers 5β-cholestanols/5β-stigmastan-3β-ol to distinguish between faeces from different sources (Bethel et al., 1994; Evershed and Bethell, 1996; Leeming et al., 1997).

In summary, the data do not support the hypothesis that 5β-cholestan-3β-ol at 30 – 40 cm soil depth in ADE originated completely from in situ reduction of cholest-5-en-3β-ol. Accordingly, the high amounts of 5β-cholestan-3β-ol at 30 – 40 cm soil depth in ADE in comparison to the reference soils fit to the hypothesis that elevated amounts of faeces were deposited on ADE in relation to the reference soils.

Correlation analyses were consistent with the interpretation that 5α-stanols and 5β-cholestan-3β-ol had different origins: Correlations between the 5α-stanols and their corresponding Δ^5-sterols fit the assumption that 5α-stanols were the product of Δ^5-sterol reduction in soils. Minor amounts of 5β-stanols were described to be a by-product of this reduction in soils. The correlation of 5β-stigmastan-3β-ol with stigmast-5-en-3β-ol as well as with 5α-stigmastan-3β-ol indicates that 5β-stigmastan-3β-ol was a product of stigmast-5-en-3β-ol reduction in ADE as well as in the reference soils and not a biomarker for the faeces of herbivore animals. A lack of such a correlation for 5β-cholestan-3β-ol underlined the idea that the 5β-cholestan-3β-ol did not completely originate from in situ cholest-5-en-3β-ol reduction, but was mainly induced by faecal deposition.

The amounts of 5β-cholestan-3α-ol (epimer of 5β-cholestan-3β-ol) correlated with the amounts of 5β-cholestan-3β-ol but also with the amounts of cholest-5-en-3β-ol and 5α-cholestan-3β-ol. Thus, the data do not show as clearly as for 5β-cholestan-3β-ol that 5β-cholestan-3α-ol did not completely originate from in situ cholest-5-en-3β-ol reduction. Corresponding with the amounts of 5β-cholestan-3β-ol, the amounts of 5β-cholestan-3α-ol were increased at 30 – 40 cm soil depth in ADE in comparison to the reference soils. In contrast, the amounts of 5β-cholestan-3α-ol were significantly elevated in ADE vs. the reference soils in the upper 10 cm and they were higher at this depth in relation to the 30 – 40 cm soil depth like the amounts of cholest-5-en-3β-ol and 5α-cholestan-3β-ol.

Bull et al. (1999b, 2002) interpreted elevated 5β-cholestan-3α-ol amounts in soils mainly by post digestive microbial reworking of 5β-cholestan-5β-ol. Enhanced microbial activity in the upper 10 cm of ADE vs. the 30 – 40 cm soil depth caused by continuous input of fresh litter probably provoked continuous microbial epimerisation of anciently introduced 5β-cholestan-3β-ol after abandonment of the ADE sites. Thus, the higher amounts of 5β-cholestan-3α-ol in the upper 10 cm vs. the 30 – 40 cm soil depth could be caused by enhanced transformation of the anciently deposited 5β-cholestan-3β-ol into

5β-cholestan-3α-ol. Additionally, the continuous input of litter in the upper 10 cm leads to higher amounts of cholest-5-en-3β-ol and 5α-cholestan-3β-ol vs. the 30 – 40 cm soil depth. In this way, the correlations of 5β-cholestan-3α-ol with cholest-5-en-3β-ol and 5α-cholestan-3β-ol could be explained by a spatial conformity of enhanced microbial epimerisation of 5β-cholestan-3β-ol and by enhanced input of litter-derived Δ^5-sterols and their in situ reduction to 5α-stanols in the upper part of the ADE soil profiles.

Though the interpretation of the 5β-cholestan-3α-ol concentrations remains speculative and accordingly the validity of 5β-cholestan-3α-ol as faecal biomarker is not as clear as the validity of 5β-cholestan-3β-ol. Including 5β-cholestan-3α-ol in the 5β /(5β + 5α)-cholestanol ratios also showed significantly higher values in ADE at 30 – 40 cm soil depth compared to the reference soils. This is concordant with the interpretation that higher amounts of faeces were deposited on ADE compared to the reference soils in the past.

The sterol patterns showed that faeces were a source for the high nutrient stocks in ADE. Faecal material is not only a nutrient rich organic material, the application of faeces to soils under agriculture also causes shifts in their microbial community structure (Enwall et al., 2007; Maeder et al., 2002; Marschner et al., 2003; Peacock et al., 2001; Sun et al., 2004). The microbial community structure of ADE differs from the microbial communities in surrounding soils (Kim et al., 2007; O'Neill et al., 2009; Ruivo et al., 2009). The deposition of faeces could have favoured this microbial community structure in ADE which is assumed to have changed soil nutrient and carbon dynamics in ways that promoted the sustainability (Lehmann, 2009). Therefore, faeces could be an important fertilizer for a sustainable agriculture in the humid tropics which imitates properties of ADE.

3.5.4 Origin of faeces in ADE

A precise identification of the faeces deposited on ADE is restrained by the lack of data about the stanol composition of faeces of soil fauna (Bethel et al., 1994) and Amazonian animals. Analyses of the biomarker patterns in the faeces from these animals including further biomarkers e.g. bile acids (Bull et al., 1999a, 1999b, 2002, 2005; Elhmmali et al., 1997; 2000; Evershed and Bethell, 1996; Knights et al., 1983; Simpson et al., 1999b; Tyagi et al., 2008) could help to identify the specific origin of this faeces. Although a precise identification of the faeces is not possible with the current data, the concentrations of different 5β-stanols in soils alone deliver hints about the origin of the faeces. 5β-cholestan-3β-ol was detected in the faeces of omnivores, carnivores and herbivores (e.g. Evershed et al., 1997; Leeming et al., 1996; Shah et al., 2007; Subbiah et al., 1972; Tyagi et al., 2008). However, 5β-cholestan-3β-ol is much lower concentrated in the faeces of carnivores (and birds) than in the faeces of omnivorous mammals (Leeming et al., 1996; Shah et al., 2007). In addition to 5β-cholestan-3β-ol, 5β-stigmastan-3β-ol has a high abundance in the faeces of herbivores (Evershed et al., 1997; Gill et al., 2010; Leeming et al., 1996; Shah et al., 2007). Based on this observation several criteria were defined to distinguish faecal inputs from herbivores vs. faeces of the omnivores, namely humans and pigs (Bethel et al., 1994; Evershed and Bethell, 1996; Leeming et al., 1997). In ADE, the absence of 5β-stigmastan-3β-ol at 30 – 40 cm soil depth in most samples showed that faeces of herbivores were not generally deposited on ADE to the same extend as the faeces of omnivores. Pigs were brought to South America by the Europeans and thus their faeces can not have contributed to the nutrient stocks in ADE. This suggests that the faecal material in ADE is of human origin.

Fertilization with human faeces was a widespread technique in pre-historic times (Denevan, 1995). However, the question whether the faecal material on ADE was intentionally applied to improve their fertility or whether the deposition of faeces was only a by-product of human activity needs further investigation. Besides the question if the faeces were intentionally applied or not, the data suggest an on-side deposition of human excrements. Therefore, faecal material should be considered as input path of nutrients into ADE when phosphor stocks and the amounts of other nutrients in ADE are used to calculate population densities in pre-Columbian Amazonia.

3.6 Conclusions

The distribution of 5α-stanols in ADE and reference soils could be explained by in situ reduction of Δ^5-sterols. In ADE, increased amounts of the faecal biomarker 5β-cholestan-3β-ol at 30 – 40 cm soil depth compared to reference soils did not correlate with increases of cholest-5-en-3β-ol and 5α-cholestan-3β-ol contents of similar magnitudes. This revealed that the elevated amounts of 5β-cholestan-3β-ol in ADE compared to the reference soils did not originate from in situ cholest-5-en-3β-ol reduction and that 5β-cholestan-3β-ol could be used as biomarker for faeces in ADE. Thus, the increased amounts of 5β-cholestan-3β-ol in ADE compared to the reference soils showed that faeces were deposited on ADE and contributed their genesis.

Faeces should be considered as nutrient source in estimations using nutrient amounts in ADE to calculate population densities in pre-Columbian Amazonia and they could be an important fertilizer for a sustainable agriculture in the humid tropics which imitates properties of ADE.

Chapter 4

Combined quantification of faecal sterols, stanols, stanones and bile acids in soils and terrestrial sediments by gas chromatography-mass spectrometry

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4.1 Abstract

Faeces incorporation can alter the concentration patterns of stanols, stanones, Δ^5-sterols and bile acids in soils and terrestrial sediments. A joint quantification of these substances would give robust and specific information about the faecal input. Therefore, a method was developed for their purification and determination via gas chromatography-mass spectrometry (GC–MS) based on a total lipid extract (TLE) of soils and terrestrial sediments. Stanols, stanones, Δ^5-steroles and bile acids were extracted by a single Soxhlet extraction yielding a TLE. The TLE was saponified with KOH in methanol. Sequential liquid–liquid extraction was applied to recover the biomarkers from the saponified extract and to separate the bile acids from the neutral stanoles, stanones and Δ^5-steroles. The neutral fraction was directly purified using solid phase extraction (SPE) columns packed with 5% deactivated silica gel. The bile acids were methylated in dry HCl in methanol and purified on SPE columns packed with activated silica gel. A mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine was used to silylate the hydroxyl groups of the stanols and Δ^5-sterols avoiding a silylation of the keto groups of the stanones in their enol-form. Silylation of the bile acids was carried out with *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing *N*-trimethylsilylimidazole (TSIM). TLEs from a set of soils with different physico-chemical properties were used for method evaluation and for comparison of amounts of faecal biomarkers analysed with saponification and without saponification of the TLE. Therefore, a Regosol, a Podzol and a Ferralsol were sampled. To proof the applicability of the method for faecal biomarker analyses in archaeological soils and sediments, additional samples were taken from pre-Columbian Anthrosols in Amazonia and an Anthrosol from a site in central Europe settled since the Neolithic. The comparison of the amounts of steroids in combination with and without saponification of the TLE showed that high amounts of faecal biomarkers occur bound to other lipids and were liberated by saponification. The method was evaluated by standard addition. The standard contained 5β-stanols, 5β-stanones and their 5α-isomers together with Δ^5-sterols and bile acids (19 substances). The standard addition revealed mean recoveries of individual substances ≥85%. The recoveries of biomarkers within each biomarker group did not differ significantly. Precisions were ≤0.22 (RSD) and quantification limits were between 1.3 and 10 ng g^{-1} soil. These data showed that the method can be applied for quantification of trace amounts of faecal steroids and for the analyses of steroid patterns to detect enhanced faeces deposition in soils and sediments.

4.2 Introduction

Faecal deposition to soils and terrestrial sediments has been traced mainly by 5β-stanols (Bethel et al., 1994; Bull et al., 2001; Hjulström and Isaksson, 2009; Simpson et al., 1998; Sweetwood et al., 2009). The potential of 5β-stanols as biomarkers to detect sewage pollution was intensively investigated in marine and limnological environments (Bull et al., 2002; Hagedorn and Weisberg, 2009; Vivian, 1986; Walker et al., 1982). Additionally, their potential was described to identify modern faeces application in agricultural soils (Ibanez et al., 2000; Jardé et al., 2007, 2009; Puglisi et al., 2003; Rogge et al., 2006). Their high stability also permits their application in an archaeological context (Bull et al., 1999b, 2002; Evershed and Bethell, 1996). Thus, they are used to detect faecal input into archaeological soils and sediments (Bull et al., 2001, 2003; Hjulström and Isaksson, 2009; Simpson et al., 1999b; Sweetwood et al., 2009). 5β-Stanones are assumed to have a potential as biomarkers comparable with 5β-stanols (Grimalt et al., 1990) but were rarely analysed in soils and terrestrial sediments (Knights et al., 1983).

Both 5β-stanols and 5β-stanones are not exclusively attributed to anthropogenic faeces, livestock breeding or manuring. Small amounts of these substances in soils could originate from faeces of wildlife and in situ reduction of Δ^5-sterols in soils (Bethel et al., 1994). To avoid misinterpretation of the concentrations of 5β-stanols and 5β-stanones, their amounts should be related to the amounts of their precursors, Δ^5-sterols, and/or their 5α-isomers (the main reduction products of Δ^5-sterols in the environment) (Bull et al., 1999b, 2002). Thus methods for faecal biomarker analysis should also allow quantification of Δ^5-sterols and 5α-stanols and/or 5α-stanones.

Although even small concentrations of 5β-stanols and/or 5β-stanones, if related to the concentrations of their precursors and 5α-isomers, allow reliable detection of enhanced faeces deposition, they provide only raw information about the origin of the faecal material (Bethel et al., 1994; Bull et al., 1999b, 2002; Evershed and Bethell, 1996; Leeming et al., 1997). Therefore, bile acids were analysed in some studies as additional biomarkers (Bull et al., 1999a, 2003; Knights et al., 1983; Simpson et al., 1999b) which deliver more detailed information about the source of faeces (Bull et al., 2002; Elhmmali et al., 1997; Evershed and Bethell, 1996; Tyagi et al., 2008). However, if solely bile acids are analysed, validation if small amounts of bile acids originate from enhanced faeces deposition or originate from natural background is not possible. This is hindered because no biomarker ratios for bile acids have been described which enable detection of enhanced faeces deposition analogous to the ratios of 5β-stanols and 5β-stanones to Δ^5-sterols, and/or their 5α-isomers. Thus, a reliable detection of enhanced faeces deposition in environmental samples combined with identification of the source of faeces demands at least the analyses of stanols and bile acids (Bull et al., 1999b, 2002; Evershed and Bethell, 1996).

The high potential of simultaneous analyses of faecal biomarkers from different groups is well described (Bull et al., 1999b, 2002; Evershed and Bethell, 1996). However, neither the development of a specific method for combined analyses of faecal biomarkers of different substance classes in soils or terrestrial sediments, nor detailed validation of the methods applied to such samples have been published, yet. Therefore, the aim of the current study was to develop a method enabling the quantification of 5β-stanols, 5β-stanones and their 5α-isomers together with Δ^5-sterols and bile acids based on a single lipid extraction (total lipid extract, TLE).

Steroids were extracted by a lipid extraction because they are usually extracted with organic solvents (Bull et al., 2002; Evershed and Bethell, 1996). In some studies the lipid extracts of samples from soils and terrestrial sediments are saponified (Bull et al., 2003; Ibanez et al., 2000; Knights et al., 1983; Simpson et al., 1999b; Sweetwood et al., 2009). However, detailed investigation of the effect with respect to such a saponification is scarce. Therefore, the amounts of free steroids were compared with the amounts of steroids bound to other lipids. For this purpose, different saponification procedures were compared to liberate bound steroids.

For separation and purification of stanols, stanones, Δ^5-sterols and bile acids, existing methods for steroid analyses in marine, limnological and biological samples were modified and combined. Bile acids were separated from neutral stanols, stanones and Δ^5-sterols by sequential liquid–liquid extraction. The neutral fraction was directly cleaned up using SPE and the bile acid fraction was achieved using SPE after methylation of the carboxyl group. Hydroxyl groups were silylated before GC–MS measurements.

The method and the applicability of potential first internal standards (IS1) were validated by standard addition to the TLEs from samples of six different soils at four concentration levels. The standard contained two 5β-stanols, three 5α-stanols, one 5β-stanone, two 5α-stanones, three Δ^5-sterols and eight bile acids (Tab. 4.1). The soil samples originated from the tropics and temperate zones with strongly different physico-chemical properties (Tab. 4.2). The texture of the samples varied from sandy texture to clayey and silty texture including loamy samples. The pH varied from acid to neutral and contents of carbon and nutrients varied as well as cation exchange capacity over a wide range. Archaeological Anthrosols were included to prove the applicability of the method for analysing faecal biomarkers in archaeological soils and sediments.

Table 4.1 Investigated compounds, retention times (RT), selected characteristic ion fragments and added amounts for method evaluation by standard addition

Biomarker group	Substance	No.	RT (min)	Chatracteristic ion fragments (m/z)	Amounts spiked to the TLE in the standard addition experiment (ng)
Stanols	5α-Pregnan-3β-ol[a;b]	1	18.7	286; 361	0; 100; 500; 1000
	5β-Cholestan-3β-ol[c]	2	27.0	215; 257; 370	0; 50; 250; 500
	5β-Cholestan-3α-ol[b]	3	27.6	215; 355; 370	0; 50; 250; 500
	5α-Cholestan-3β-ol[d]	4	29.7	217; 445; 460	0; 100; 500; 1000
	5α-Stigmastan-3β-ol[c]	5	35.1	215; 383; 398	0; 200; 1000; 2000
Stanones	5α-Pregnan-3-one[e, b]	6	18.9	217; 302	0; 100; 500; 1000
	5β-Cholestan-3-one[b]	7	29.0	231; 386	0; 100; 500; 1000
	5α-Cholestan-3-one[c]	8	30.3	231; 386	0; 100; 500; 1000
Δ^5-Sterols	Cholest-5-en-3β-ol[c]	9	29.4	329; 368; 458	0; 400; 2000; 4000
	Stigmasta-5,22-dien-3β-ol[f]	10	33.2	255; 394; 484	0; 1000; 5000; 10000
	Stigmast-5-en-3β-ol[g]	11	35.2	357; 396; 486	0; 1000; 5000; 10000
Bile acids	7α,12α-Dihydroxy-5β-cholanoic acid (IDCA)[e, b]	12	26.1	255; 355; 370	0; 100; 500; 1000
	3β-Hydroxy-5β-cholanoic acid (ILCA)[b]	13	28.8	215; 257; 357	0; 50; 250; 500
	3α-Hydroxy-5β-cholanoic acid (LCA)[f]	14	29.9	215; 257; 372	0; 100; 500; 1000
	3β,12α-Dihydroxy-5β-cholanoic acid (BDCA)[b]	15	30.1	255; 345	0; 50; 250; 500
	3α,12α-Dihydroxy-5β-cholanoic acid (DCA)[c]	16	31.0	255; 345; 370	0; 200; 1000; 2000
	3α,7α-Dihydroxy-5β-cholanoic acid (CDCA)[c]	17	31.8	255; 355; 370	0; 100; 500; 1000
	3α,6α-Dihydroxy-5β-cholanoic acid (HDCA)[b]	18	32.4	255; 355; 370	0; 100; 500; 1000
	3α,7β-Dihydroxy-5β-cholanoic acid (UDCA)[f]	19	33.6	255; 370; 460	0; 100; 500; 1000
Second internal standard[h]	5α-Cholestane[c]	20	23.2[i]; 21.5[j]	217; 357; 372	

[a] First internal standard for stanols and Δ^5-sterols

[b] Obtained from Steraloids (Newport, RI, USA)

[c] Obtained from Sigma-Aldrich (St. Louis, MO, USA)

[d] Obtained from Alfa Aesar (Ward Hill, MA, USA)

[e] First internal standard for the biomarker group

[f] Obtained from Fluka (St. Louis, MO, USA)

[g] Obtained from ChromaDex (Irvine, CA, USA)

[h] All measurements

[i] Measurement of stanols, stanones, Δ^5-sterols

[j] Measurement of bile acids

Table 4.2 Physico-chemical characterisation of the soil samples.

Location	Soil[a]	Depths (cm)	Texture[b]	pH (KCl)[c]	pH (H_2O)[c]	C (organic) (%)	N (total) (%)	CEC (pot) (cmol$_{c+}$ kg^{-1})[d]	Al (avail.) (mg kg^{-1})[e]	Ca (avail.) (mg kg^{-1})[e]	K (avail.) (mg kg^{-1})[e]	Mg (avail.) (mg kg^{-1})[e]	Na (avail.) (mg kg^{-1})[e]	P (avail.) (mg kg^{-1})[e]
Amazonia (Brazil)	Anthrosol	0–50	Clay (heavy clay)	6.0	5.2	5.31	0.33	29	1050	2991	31	451	5	42
	Ferralsol	0–50	Clay (heavy clay)	3.6	3.8	1.22	0.11	6	1040	24	11	6	2	4
Amazonia (French Guiana)	Anthrosol	0–80	Sand	4.0	5.4	0.65	<0.05	1	588	281	65	11	2	244
	Podzol	0–80	Sand	3.3	4.5	0.38	<0.05	0	47	38	8	13	3	3
Central Europe (Germany)	Anthrosol	0–70	Loam	4.8	6.3	2.09	0.19	37	950	5896	143	965	14	62
	Regosol	0–70	Silt loam	4.9	6.5	0.50	0.05	14	1036	2605	187	302	8	51

[a] Reference soil group of the WRB (IUSS Working Group WRB, World Reference Base for Soil Resources 2006, FAO, Rome, 2nd ed., 2006.)

[b] Textural class of the WRB (FAO, Guidelines for soil description, FAO, Rome, 4th ed., 2006.)

[c] Analysed according to the method of ISRIC (L.P. van Reeuwijk (Ed.), Procedures for Soil Analysis, ISRIC, Wageningen, 6th ed., 2002.)

[d] Potential cation exchange capacity at pH 7 analysed according to the method of ISRIC with ammonuim acetate (L.P. van Reeuwijk (Ed.), Procedures for Soil Analysis, ISRIC, Wageningen, 6th ed., 2002.)

[e] Plant available amounts analysed by Mehlich No. 3 extraction (R. Burt (Ed.), Soil Survey Laboratory Methods Manual, NRCS, Lincoln, 4th ed., 2004.)

4.3 Experimental

4.3.1 Chemical and reagents

Standards of stanols, stanones, Δ^5-sterols and bile acids are listed in Table 4.1. They were purchased from different suppliers which are indicated in Table 4.1. Dry 1.25 M HCl in methanol, *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) and *N*-trimethylsilylimidazole (TSIM) were obtained from Sigma-Aldrich (St. Louis, MO, USA) and a mixture of hexamethyldisilazane (HMDS), trimethylchlorosilane (TMCS) and pyridine (3:1:9, v/v/v, Sylon HTP) was obtained from Supelco (St. Louis, MO, USA). Distilled water was used and all other chemicals were at least analytical grade and were purchased from various suppliers.

Not included in this study were 5β-stigmastan-3β-ol and 5β-stigmastan-3-one (biomarker indicative for the faeces of herbivores) because standards were not available.

4.3.2 Soil samples

Six different soils were used to validate the method (Tab. 4.2). One Anthrosol (*terra preta de Índio*) and one adjacent Ferralsol were sampled in Brazil in Amazonia in the west of Pará. One Anthrosol and one adjacent Podzol were collected in north western French Guiana and one Anthrosol and one adjacent Regosol were sampled in Germany in the north of Bavaria. The Anthrosols in South America is of pre-Columbian origin and the Anthrosol in Germany was taken in an area which has been settled since the Neolithic. The whole topsoil material was sampled and combined to one sample for each Anthrosol. The corresponding depths of the adjacent soils were used, respectively. The exact sampling depths and physico-chemical properties of the samples are given in Table 4.2.

The soil samples were dried at 40 °C, sieved <2 mm and finely ground in a ball mill.

4.3.3 Lipid extraction

Samples were extracted via Soxhlet extraction according to a slightly modified method which was previously used to yield a TLE including steroids (Bull et al., 2003; Simpson et al., 1999b; Sweetwood et al., 2009). 5 g of soil samples from Anthrosols and 10 g of soil samples from the other soils were weighted into 23 mm × 100 mm fibreglass thimbles (Macherey-Nagel, Düren, Germany) and covered by a thin layer of quartz sand (fibreglass thimbles, quartz sand and all glass ware were heated at 300 °C for 12 h in a muffle furnace prior to use). Extraction was done with 150 mL dichloromethane/methanol (2:1, v/v) for 36 h. After extraction, the solution was concentrated by rotary evaporation and completely dried under a gentle stream of nitrogen. The drying under a gentle stream of nitrogen was always done to dry extracts and fractions and is therefore not repeatedly described in the following sections of the method description.

4.3.4 Saponification

A procedure was slightly modified which was introduced by Grimalt et al. (1990) for the combined analyses of stanols, stanones and Δ^5-sterols in water particulate and sediment samples. The dried extract was dissolved in 3.5 mL 0.7 M KOH in methanol and reaction was allowed overnight (10-14 h) at room temperature. Figure 4.1 summarises the saponification and the purification of stanols, stanones, Δ^5-sterols and bile acids in the TLE.

4.3.5 Sequential liquid–liquid extraction

The sequential liquid–liquid extraction is based on methods to analyse steroids in faeces (Batta et al., 1999; Grundy et al., 1965), but was strongly modified. 10 mL water was added to the saponified extract and the neutral fraction including the stanols, stanones and Δ^5-sterols was separated by repeated liquid–liquid extraction with chloroform (3× 15 mL). To extract the bile acids, 6 M HCl was added to the saponification/water solution for acidification (pH ≤ 2) and the acidic fraction including the bile acids was extracted by repeated liquid–liquid extraction with chloroform (3× 15 mL). The chloroform extracts were concentrated by rotary-evaporation and dried, thereafter.

4.3.6 Methylation of bile acids

The bile acids were methylated based on combination of different methods (Batta and Salen, 1999; Begerow and Dunemann, 1998.). 1 mL dry 1.25 M HCl in methanol were added to the acidic fraction and kept at 80 °C for 2 h. The methyl esters were extracted after addition of 1 mL water by repeated liquid–liquid extraction with hexane (3× 1 mL), followed by drying of the extract.

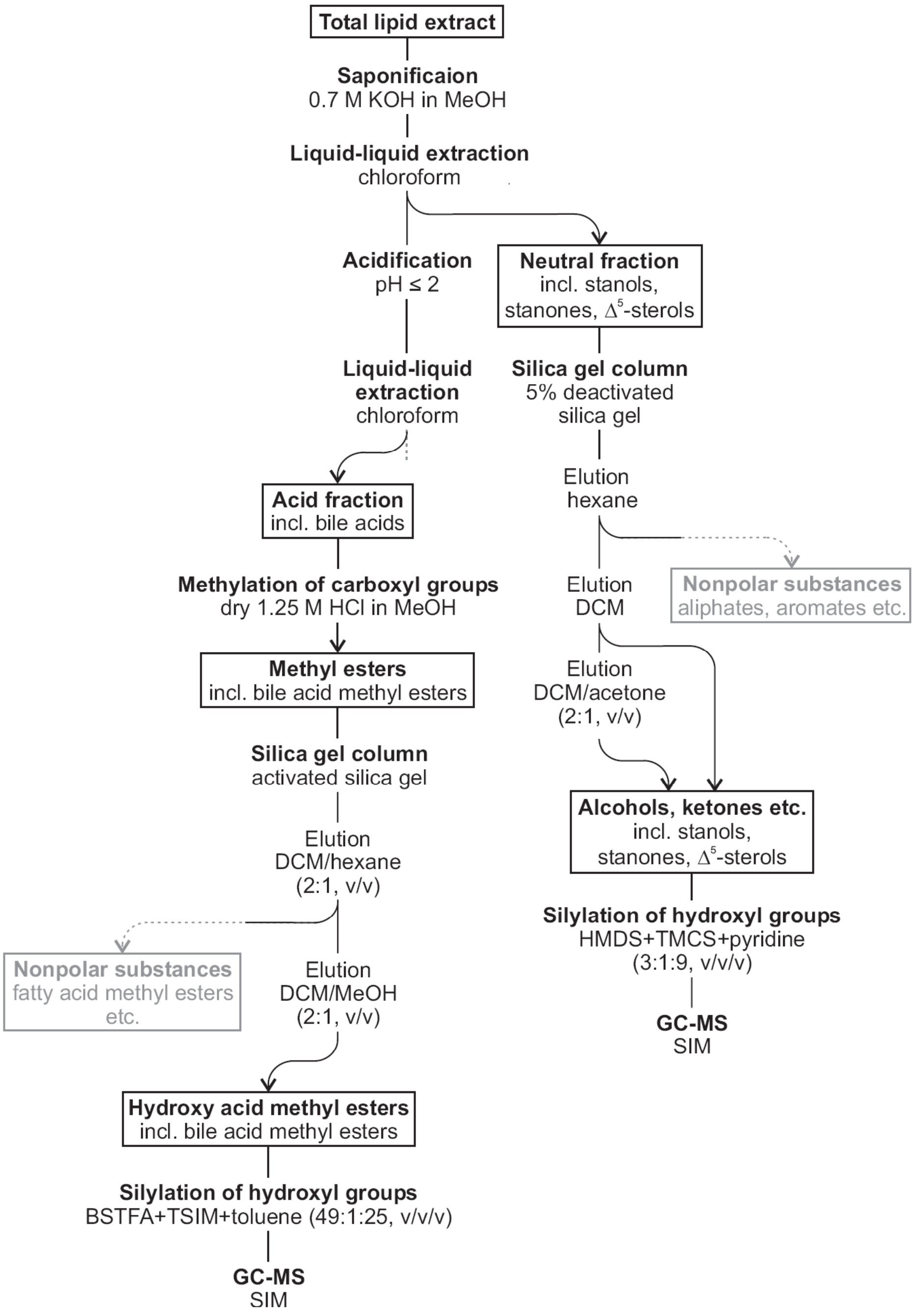

Figure 4.1 Schematic diagram of the analytical procedure. MeOH: methanol, DCM: dichloromethane, HMDS: hexamethyldisilazane, TMCS: trimethylchlorosilane, BSTFA: *N,O*-bis(trimethylsilyl)trifluoroacetamide, TSIM: *N*-trimethylsilylimidazole, GC–MS: gas chromatography-mass spectrometry, SIM: selected ion monitoring mode)

4.3.7 SPE clean up

4.3.7.1 SPE clean up of stanols, stanones and Δ^5-sterols

A solid phase-solvent combination initiated by Isobe et al. (2002) for cleaning up river sediment and water samples was optimized. Columns (5 mm diameter, PE) packed with 5% deactivated silica gel (50 mm, mesh: 70–230, pore size 100 Å, type: Merck 10187, Sigma-Aldrich, St. Louis, MO, USA) were preconditioned with hexane. The fraction containing stanols, stanones and Δ^5-sterols was transferred onto the columns with hexane. Less polar substances (aliphatic and aromatic compounds etc.) were eluted with 5 mL hexane and discarded. The fraction containing both, ketones and alcohols including stanones, stanols and Δ^5-sterols, was eluted together without a separation of stanones from stanols and Δ^5-sterols. This fraction was eluted first with 3 mL dichloromethane and afterwards with 2 mL dichloromethane/acetone (2:1, v/v). The dichloromethane and dichloromethane/acetone eluates were combined and dried.

4.3.7.2 SPE clean up of bile acid methyl esters

The method used by Elhmali et al. (2000) to clean up bile acids extracted from water samples was modified. The fraction containing bile acid methyl esters was transferred with hexane onto columns (5 mm diameter, PE) packed with activated silica gel (50 mm, mesh: 70–230, pore size 100 Å, type: Merck 10187, Sigma-Aldrich, St. Louis, MO, USA) preconditioned with dichloromethane/hexane (2:1, v/v). Less polar substances (fatty acid methyl esters, etc.) were eluted with 5 mL dichloromethane/hexane (2:1, v/v). The hydroxy acids including bile acids were eluted with 5 mL dichloromethane/methanol (2:1, v/v) and the eluate was dried.

4.3.8 Silylation

4.3.8.1 Silylation of stanols and Δ^5-sterols

The fraction containing stanols, stanones and Δ^5-sterols was heated in 100 µL of a mixture of HMDS, TMCS and pyridine (3:1:9, v/v/v) at 70 °C for 1 h (Batta et al., 2002; Elhmmali et al., 2000; Ibanez et al., 2000; Korpela et al., 1986; Setchell et al., 1983). Excess silylation reagent was evaporated. 20 ng 5α-cholestane (dissolved in dry toluene, 20 µL, 0.001 µg μL^{-1}) were added as second internal standard (IS2) (Bethel et al., 1994;

Bull et al., 2001; Evershed et al., 1997; Osman et al., 2008; Van Bergen et al., 1997). Before the measurement, 100 µL dry toluene were added.

4.3.8.2 Silylation of bile acids

The fraction containing bile acids was re-dissolved in 50 µL dry toluene and 98 µL BSTFA and 2 µL TSIM were added. After heating at 80 °C for 1 h, 20 ng 5α-cholestane (dissolved in dry toluene, 20 µL, 0.001 µg μL^{-1}) were added as IS2 (Bethel et al., 1994; Bull et al., 2001; Evershed et al., 1997; Osman et al., 2008; Van Bergen et al., 1997).

4.3.9 External standards

Solutions of the relevant biomarkers, as far as commercially available, and solutions of IS1 (5α-pregnan-3β-ol, 5α-pregnan-3-one and 7α,12α-dihydroxy-5β-cholanoic acid) were prepared in methanol and derivatized using the same methods, which were used for the soil extracts. In Table 4.1, the standard substances are listed.

4.3.10 GC–MS analysis and quantification

All substances were analysed via gas chromatography-mass spectrometry (GC–MS) with a HP 5971A quadrupole mass spectrometer (Hewlett-Packard, Palo Alto, CA, USA) connected to a HP 5890 gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA). A DB-5 MS (122-5532, Agilent, Santa Clara, CA, USA), 30 m fused silica column with 0.25 mm I.D. and 0.25 µm film thickness was directly coupled with the mass spectrometer. He (99.9990% purity) was used as carrier gas at 0.8 bar constant pressure. The injection port was set to 250 °C and 1 µL was injected in splitless mode (1 min splitless time) via a splitless liner (5062-3587, Agilent, Santa Clara, CA, USA). For analyses of Δ^5-sterol derivatives, the solution containing the stanol derivatives, the stanones and the Δ^5-sterol derivatives was diluted by 1:5 after measurement of the stanols and stanones. The transfer line temperature was 280 °C. The solvent delay was 14 min and electron ionisation was used at 70 eV. Measurements in scan mode were done to verify peak identity and measurements in selected ion monitoring mode (SIM) were performed for quantification (Tab. 4.1 shows the selected ions).

For analyses of stanol derivatives, stanones and Δ^5-sterol derivatives, the column temperature programme was 80 °C (held 1.5 min) to 265 °C at 12 °C min^{-1}, to 288 °C at 0.8 °C min^{-1} and to 300 °C (held 12 min) at 10 °C min^{-1}.

For analyses of bile acid derivatives, the column temperature programme was 80 °C (held 1.5 min) to 250 °C at 20 °C min^{-1}, to 287 °C at 1.2 °C min^{-1} and to 300 °C (held 12 min) at 10 °C min^{-1}.

For quantification, peak areas of the analytes in the samples and the substances in the external standards were divided by the peak area of the IS2, respectively. Using these ratios, calibration curves for each substance were calculated from the data of the external standards.

4.3.11 Comparison of effects of different saponification procedures

Three replications of each soil sample were extracted according to the procedure described in Section 4.3.3. The TLE of one replicate was saponified using the procedure described in Section 4.3.4. For another replicate more rigorous saponification conditions were tested. For this purpose, the dried extract was dissolved in 3.5 mL 5 M KOH in 90% methanol and the solution was heated to 120 °C for 1 h (Bull et al., 2003; Simpson et al., 1999b). The third replicate was analysed without saponification of the TLE. Purification, derivatisation and GC–MS measurements were done as described in Sections 4.3.5-4.3.10.

For this comparison, 500 ng of each IS1 were added directly to the soil samples before the extraction and the recoveries of the IS1 were used to correct for losses of analytes during the analyses.

4.3.12 Method evaluation

4.3.12.1 Standard addition

To validate the method by standard addition, the same standard solutions were used which served as external standards and IS1 (Section 4.3.9 and Tab. 4.1). Each soil sample was extracted in four replications as described in Section 4.3.3 and the standard solutions were added to the TLEs. The amounts of added biomarkers were adjusted to add approximately 1.5 times, 3 times and 6 times of the expected concentrations in soils. However, attention was paid to enable measurements of all substances within each biomarker group within the linear range of the GC–MS without changing the settings. One sub-sample of each soil was analysed without standard addition. Saponification, purification, derivatisation and GC–MS measurements were done as described in Sections 4.3.4-4.3.10.

4.3.12.2 Calculation of the recovery by the standard addition method and statistical evaluation

The data of the standard addition experiment (Section 4.3.12.1) were used to calculate the mean recovery of each analyte and of the IS1. The measured concentrations of each substance were plotted vs. the added amount of standard. At first, regression lines were calculated by least square fitting for each soil separately. Thereafter, the y-intercept of the corresponding regression line (which was caused by the original concentration in the soil before standard addition) was subtracted from each value and the data of all soils were pooled. The mean recovery of each substance was calculated by determining the slope of the regression lines fitted to their pooled data.

For statistical evaluation GraphPad Prism 5 (Version 5.03, GraphPad Software Inc., San Diego, CA, USA) was used. Outliers were identified by a method proposed by Motulsky and Brown (2006) and were excluded only if an exceptional error during the analysis was recognized. The significance of the regression was tested and the Steven's Runs Test was calculated to test for significant deviations from linearity. To test for significant differences between the recoveries (slopes of regression lines) within the biomarker groups (stanols, stanones, Δ^5-sterols and bile acids, respectively) including the corresponding IS1 covariance analyses (ANCOVA) were performed according to Zar (1984). The level of significance was 0.05 for all tests.

4.3.12.3 Determination of the background values of IS1 by the standard addition method

The background values of the IS1 were calculated from the data of the standard addition experiment (Section 4.3.12.1). The x-intercepts of the regression lines, which were fitted for each soil separately (Section 4.3.12.2), were calculated as background values.

4.3.12.4 Calculation of relative standard deviation (RSD)

The RSDs were calculated from the data of the standard addition experiment (Section 4.3.12.1). The recoveries of the IS1 were used to correct for losses of analytes during the analyses. Therefore, only data of samples were used where the amount of added IS1 exceeded at least 4 times the amount, which was found in the samples by the standard addition method (Section 4.3.12.3). This was done to minimize errors due to the original presence of these substances before standard addition.

(The equation by which the RSDs were calculated is provided in the supplementary data, Appendix 9.1.)

4.3.12.5 Estimation of limits of quantification and detection

Limit of detection (LOD) was determined as signal-to-noise ratio of 3:1 and the limit of quantification (LOQ) was determined as signal-to-noise ratio of 10:1.

4.4 Results and discussion

4.4.1 Saponification

After ’mild‘ saponification of the TLEs (0.7 M KOH in methanol overnight at room temperature) up to 9 times higher amounts of steroids were measured compared to analyses without saponification. When the results of all samples and all substances were pooled together, the amounts of steroids measured in the ’mild‘ saponified TLEs were in 75% of all cases higher than their amounts measured without saponification (included in this number are the data from samples where some substances were only quantifiable in the saponified TLE; Fig. 4.2a and Fig. 4.2b). A high variability of the bound proportions was found between and within the samples and no correlations with the different sources of the biomarkers, e.g. faeces vs. other input pathways, were determined.

In principal, relative low proportions of the bile acids were bound to other lipids. Only 62% of the bile acids were measured in higher amounts in the saponified TLEs than in TLEs not saponified. In contrast, 71% of the stanols and 94% of the Δ^5-sterols were found in higher amounts in the saponified TLEs compared to TLEs not saponified. Both stanones, 5β-cholestan-3-one and 5α-cholestan-3-one, were found in all samples in the ’mild‘ saponified TLEs in concentrations above the LOQ (Fig. 4.2a). However, only in two samples free 5α-cholestan-3-one and in one sample free 5β-cholestan-3-one were found in concentrations above the LOQ (Fig. 4.2b). The higher number of bound alcohols in comparison to the bound bile acids and the abundance of bound stanones in the TLEs

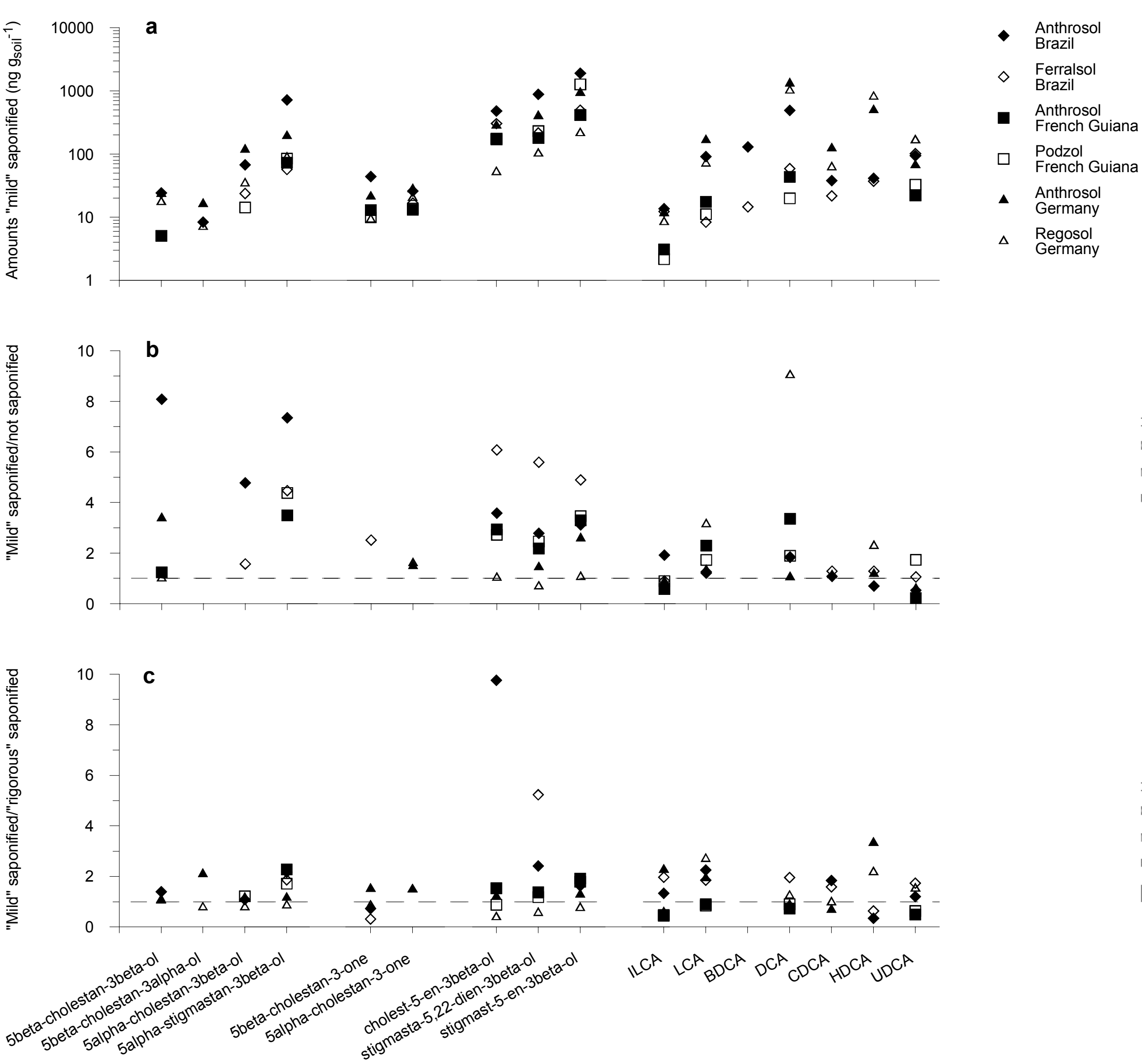

Figure 4.2 Effects of different saponification procedures of the total lipid extract (TLE). (a) Amounts analysed with a 'mild' saponification of the TLE (0.7 M KOH in methanol overnight at room temperature). (b) Ratio of the amounts analysed with a 'mild' saponification of the TLE divided by the amounts analysed without saponification of the TLE and (c) Ratio of the amounts analysed with a 'mild' saponification divided by the amounts analysed with a 'rigorous' saponification (5 M KOH in methanol, 120 °C, 1 h.) of the TLE.

question, if the steroids are bound to other lipids only by covalent bonds (Gobe and al., 2000; Jambu, 1993; Lichtfouse, 1998; Naafs and Van Bergen, 2002). Despite ketones can form ester bonds in the form of enol tautomers, also physical entrapping in lipid complexes and stabilization by hydrophobic interactions (Grasset and Amblès, 1998; Lichtfouse, 1998; Naafs and Van Bergen, 2002; Piccolo, 2002; Sutton and Sposito, 2005) could be responsible for the higher yields of steroids after saponification. Especially in the case of relatively hydrophobic stanones, the saponification of ester bonds in the TLE enhances the polarity of several lipids in the TLE. This polarity enhancement could cause liberation of molecules which were bound by hydrophobic interactions in lipid complexes.

In general, TLE saponification yields a better quantification of steroids than the analysis of free lipids. It is possible that the steroids bound to other lipids are stabilized in soils and in terrestrial sediments to a certain degree. Especially with respect to archaeological questions, it could be of interest to include steroids which are possibly stabilized. Therefore, the saponifaction step was introduced in this method. However, it may depend on the specific questions if only the analyses of free lipids or a more complete extraction might be of interest, e.g. recent vs. old land use practises.

The ’mild‘ saponification procedure was applied by Grimalt et al. (1990) for the analyses of stanols, stanones and Δ^5-sterols in water particulate and sediment samples, previously. For the analyses of bile acids in soils often more rigorous saponification conditions (5 M KOH in methanol, 120 °C, 1 h) were used (Bull et al., 2003; Simpson et al., 1999b). However, formations of artefacts are possible when stanones are exposed to rigorous saponification conditions (Isobe et al., 2002; Keller and Jahreis, 2004). A loss of stanones was also observed in this study when the rigorous saponification conditions (5 M KOH in methanol, 120 °C, 1 h.) were tested (Fig. 4.2a and Fig. 4.2c). Experiments with standard solutions containing only stanones additionally showed that Δ^5-sterols and Δ^4-sterols were formed from stanones when the 5 M KOH in methanol was prepared without water (120 °C, 1 h). A loss of stanones was also observed when the solutions of pure stanones were exposed to 5 M KOH in 90% methanol (120 °C, 1 h).

Furthermore, comparison of the ’rigorous‘ and the ’mild‘ methods did not show generally higher yields after the ’rigorous‘ saponification (Fig. 4.2c) and therefore the ’mild‘ saponification is suggested as best extraction compromise.

4.4.2 Sequential liquid–liquid extraction

Stanols, stanones and Δ^5-sterols were extracted before acidification of the KOH solution by liquid–liquid extraction with chloroform (neutral fraction). Bile acids were extracted by a second liquid–liquid extraction after acidification (acid fraction). This sequential liquid–liquid extraction is a strongly modified variant of methods to separate bile acids from other steroids which were introduced to analyse steroids in faeces (Batta et al., 1999; Grundy et al., 1965). Pre-test with pure standard solutions showed that neither bile acids were detectable in the neutral fraction nor stanols, stanones or Δ^5-sterols appeared in the acid fraction.

A separation of bile acids from other steroids was also proposed for the analyses of 5β-stanols, Δ^5-sterols and bile acids in limnic samples (Elhmmali et al., 2000). It facilitates further purification and GC–MS measurements. In this way, a clear peak separation of silyl derivatives of stanols and Δ^5-sterols from silyl derivatives of bile acids is very difficult. The same problem arises if the carboxyl group of bile acids is methylated. Only an esterification of the carboxyl groups with butanol or higher homologues of alcohols would allow a separation by GC of bile acid ester silyl derivatives from the silyl derivatives of stanols and Δ^5-sterols (Batta et al., 1999, 2002).

For extraction and separation of stanols and bile acids from biological matrixes the use of reversed phase columns is described (e.g. C_{18}) (Schüßeler et al., n.d.). For analyses of limnic samples a SPE procedure with weak cation exchange resin was proposed to separate acids from stanols and Δ^5-sterols after a joint liquid–liquid extraction of these substances (Elhmmali et al., 2000).

Both a joint GC measurement of stanols, Δ^5-sterols and bile acids and a separation of bile acids from other steroids by SPE require that the KOH solution is acidified to protonate the bile acids before the steroids are extraction. Pre-tests showed that stanones were transformed by acid-catalysed reactions (e.g. formation of cholestane-3,5-diol) when the KOH solution was acidified. The sequential liquid–liquid extraction applied in this study allows an extraction of stanones together with stanols and Δ^5-sterols before acidification of the KOH solution. This avoids a transformation of stanones. At the same time this method yields a separation of bile acids from other steroids.

4.4.3 SPE clean up and derivatisation

4.4.3.1 SPE clean up of stanols, stanones and Δ^5-sterols

A solid phase solvent combination which was introduced by Isobe et al. (2002) for cleaning up river sediment and water samples enabling also purification of stanols, stanones and Δ^5-sterols in soil samples (Fig. 4.3a and Fig. 4.3b). In contrast to the method of Isobe et al. (2002), smaller columns (5 mm diameter, 50 mm fill level instead of 10 mm diameter, 150 mm fill level) filled with 5%-deactivated silica with 70–230 mesh and 100 Å pore size were used. These smaller columns enabled the use of reduced volumes of hexane for eluting the less polar fraction as well as 3 mL instead of 40 mL dichloromethane and 2 mL instead of 30 mL dichloromethane/acetone to recover the ketone and alcohol fraction. The small solvent volumes enabled a collection of the eluates containing the biomarkers directly within 5 mL derivatisation vials, followed by a direct evaporation under a nitrogen stream. Thus, neither additional transfer of fractions nor rotary evaporation was necessary before derivatisation and only few amounts of solvents were consumed.

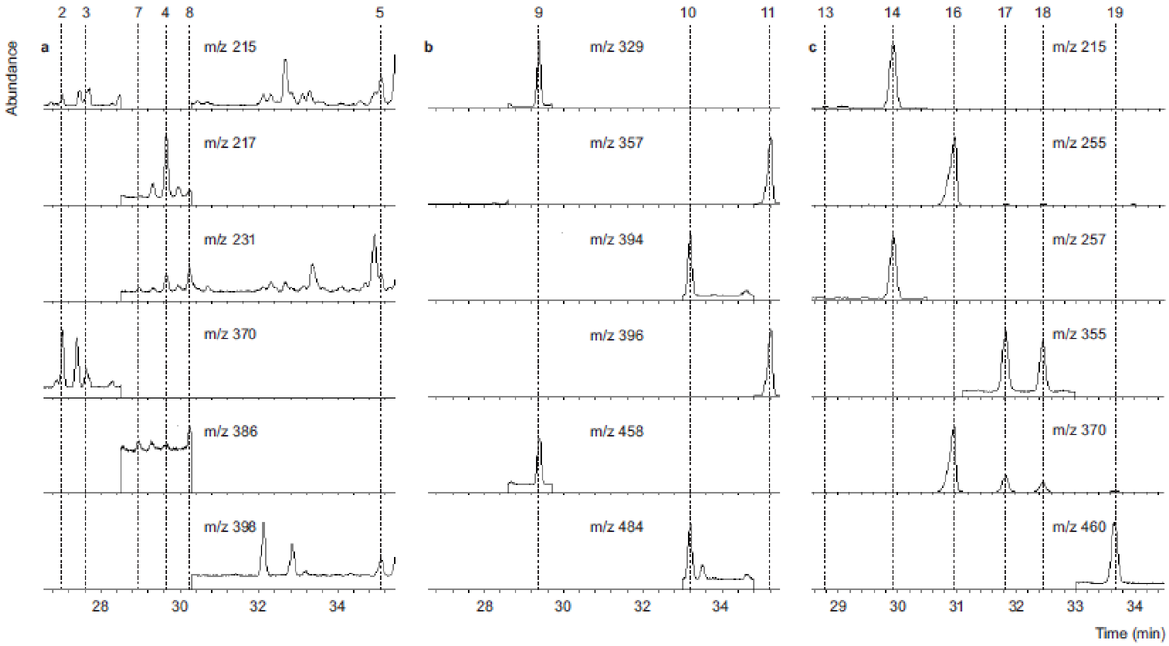

Figure 4.3 Ion chromatograms of (a) stanols and stanones, (b) Δ^5-sterols and (c) bile acids extracted from the German Anthrosol and measured in selected ion monitoring mode. Peak numbers refers to substances in Table 4.1.

This SPE procedure does not allow a separation of stanones from Δ^5-sterols and stanols. A separation of these biomarkers was not intended, because the peaks of stanones and the silyl derivates of stanols and Δ^5-sterols were well separated in a combined GC–MS measurement (Fig. 4.3a and Fig. 4.3b). A further separation of stanols and Δ^5-sterols from *n*-alkanols (Bull et al., 1998, 1999a; Simpson et al., 1998, 1999b; Sweetwood et al., 2009) as well as stanones from *n*-alkanones may be necessary for GC–FID measurements but was not required for analyses by GC–MS in this study.

4.4.3.2 Methylation of bile acids and SPE clean up of bile acid methyl esters

Bile acids were separated from long chain fatty acids by SPE with activated silica. Before SPE, their carboxyl groups were methylated with HCl in dry methanol. After methylation, bile acids methyl esters (and other hydroxy acid methyl esters) are stronger retained by the silica via their hydroxyl groups than the less polar fatty acid methyl esters. The long chain fatty acid methyl esters were eluted with dichloromethane/ hexane (2:1, v/v) and the bile acid methyl esters with dichloromethane/methanol (2:1, v/v).

Pre-experiments showed that the methylation with HCl in dry methanol, which was described in biological studies (Batta and Salen, 1999), is less effective than a methylation with diazomethane, which was often used in archaeological studies (Bull et al., 1999a, 2003; Simpson et al., 1999b). Furthermore, the methylation of the tri-hydroxy bile acid cholic acid caused severe problems. However, due to the high toxicity of diazomethane, methylation with HCl in dry methanol was preferred and cholic acid was excluded from further experiments in this study.

The SPE method based on a method which was described by Elhmali et al. (2000) to clean up bile acids extracted from water samples. Fatty acid methyl esters with chain length ≤19 are not quantitatively separated from the bile acids by this method. However, the retention times of these fatty acid methyl esters are shorter than the retention times of the bile acid methyl ester derivatives and peaks were well separated by subsequent gas chromatography (Fig. 4.3c).

4.4.3.3 Silylation of hydroxyl groups

A mixture of HMDS, TMCS and pyridine (3:1:9, v/v/v) was used for the derivatisation of the relatively unhindered hydroxyl groups of the stanols and the Δ^5-sterols. This silylation mixture was proposed frequently as silylating reagent for these substances (Batta et al., 2002; Elhmmali et al., 2000; Ibanez et al., 2000; Korpela et al., 1986; Setchell et al., 1983).

For the bile acid fraction, the stronger silylation mixture BSTFA and TSIM (98:2, v/v) was used. Due to his small molecular size TSIM is the strongest silylation reagent for hydroxyl groups and useful for the derivatisation of hindered hydroxyl groups occurring in bile acids.

In theory, BSTFA containing TSIM can also be used for the derivatisation of stanols and Δ^5-sterols. However, the keto groups of the stanones were partly derivatisised in their enol-forms when this derivatisation mixture was applied to the fraction containing the stanols, Δ^5-sterols and stanones. A more complete silylation of the stanones can be achieved using a mixture of MSTFA, TSIM, dichloromethane and triethylamine (Benfenati et al., 1994; Chou and Liu, 2004). However, Chou and Liu (2004) described the formation of Δ2 and Δ3 isomers causing two peaks for one stanone. Furthermore, a low thermo-stability of the enol-silyl derivatives of ketones was assessed (Begerow and Dunemann, 1998). Therefore, a mixture of HMDS, TMCS and pyridine was used for the silylation of the stanols and the Δ^5-sterols in this study, which did not silylate stanones.

4.4.4 Method validation and analytical performance

4.4.4.1 Linearity determined by the standard addition method

After subtraction of the y-intercept of the regression lines calculated from the data of the standard addition for each substance and each sample separately, the data of each substance scattered around a mean regression line. These mean regressions were significant and no significant deviation from linearity was detected using the Steven's Runs test for large concentration ranges (Tab. 4.3).

Most substances had regression coefficients ≥0.85. Regression coefficients in the range of 0.80–0.85 were measured only for 5α-stigmastan-3β-ol and for 3α-hydroxy-5β-cholanoic acid (LCA), 3α,6α-dihydroxy-5β-cholanoic acid (HDCA) and 3α,7β-dihydroxy-5β-cholanoic acid (UDCA). For these substances the ratios of added standard vs. the original concentrations in the samples before/without standard addition were relatively low (Tab. 4.1 and Fig. 4.2a). Thus, relatively low deviations in extraction efficiency before standard addition could have caused high deviation from the mean regression line. This effect could have been strongly pronounced for 3α,12α-dihydroxy-5β-cholanoic acid (DCA). For this bile acid the ratio of added standards vs. concentration in the samples before standard addition was very low. This likely caused the exceptional low regression coefficient (0.69) of DCA.

4.4.4.2 Recovery determined by the standard addition method

The slopes of the mean regression lines, calculated from the data of the standard addition experiment, were in a range of 0.85–1.18. Thus, the deviations of the mean recoveries from 100% recovery were ≤18% showing that all substances can be quantified with a high recovery.

By covariance analyses, no significant differences were detected between the recoveries of the individual substances within the stanols, the stanones, the Δ^5-sterols and the bile acids including the corresponding IS1, respectively. This indicates that the biomarker patterns within each biomarker group were not systematically altered by the method. Accordingly, these data are in agreement with an application of the method to analyse the deposition of faeces and the origin of faeces by biomarker ratios (Bull et al., 1999b, 2002).

Table 4.3 Regression coefficients (R^2), recovery (± standard error) and relative standard deviation (RSD) calculated from data of standard addition to soil samples and limit of detection (LOD) and limit of quantification (LOQ).

Biomarker group	Substance	R^2	Recovery (%)	RSD	LOD (ng g_{soil}^{-1})	LOQ (ng g_{soil}^{-1})
Stanols	5α-Pregnan-3β-ol[a]	0.91	91 ± 6	-	2.5	5.0
	5β-Cholestan-3β-ol	0.94	118 ± 6	0.18	0.7	1.3
	5β-Cholestan-3α-ol	0.91	104 ± 7	0.11	1.0	1.4
	5α-Cholestan-3β-ol	0.91	99 ± 7	0.14	3.0	8.0
	5α-Stigmastan-3β-ol	0.84	110 ± 10	0.12	5.0	7.5
Stanones	5α-Pregnan-3-one[b]	0.91	114 ± 8	-	2.5	4.0
	5β-Cholestan-3-one	0.86	108 ± 9	0.07	3.0	4.2
	5α-Cholestan-3-one	0.85	102 ± 9	0.16	2.5	5.0
Δ^5-Sterols	Cholest-5-en-3β-ol	0.97	108 ± 4	0.22	4.0	10.0
	Stigmasta-5,22-dien-3β-ol	0.95	104 ± 5	0.22	4.0	7.0
	Stigmast-5-en-3β-ol	0.95	106 ± 5	0.19	2.0	7.0
Bile acids	7α,12α-Dihydroxy-5β-cholanoic acid (IDCA)[b]	0.96	94 ± 4	-	1.9	2.6
	3β-Hydroxy-5β-cholanoic acid (ILCA)	0.98	114 ± 3	0.15	0.5	1.3
	3α-Hydroxy-5β-cholanoic acid (LCA)	0.84	85 ± 8	0.09	0.5	2.0
	3β,12α-Dihydroxy-5β-cholanoic acid (BDCA)	0.91	93 ± 6	0.08	1.8	3.0
	3α,12α-Dihydroxy-5β-cholanoic acid (DCA)	0.69	106 ± 15	0.12	1.8	5.0
	3α,7α-Dihydroxy-5β-cholanoic acid (CDCA)	0.90	105 ± 7	0.11	2.8	4.5
	3α,6α-Dihydroxy-5β-cholanoic acid (HDCA)	0.84	87 ± 8	0.19	4.0	10.0
	3α,7β-Dihydroxy-5β-cholanoic acid (UDCA)	0.80	100 ± 11	0.14	3.0	10.0

[a] First internal standard for the stanols and Δ^5-sterols

[b] First internal standard for the biomarker group

4.4.4.3 Background values of IS1 determined by the standard addition method

5α-Pregnan-3β-ol was found in most soil samples. However, only in two soil samples concentration was >2 ng g^{-1}. In the Podzol from French Guiana it was found in a concentration of 12 ng g^{-1} and in the German Anthrosol in a concentration of 9 ng g^{-1}. 5α-Pregnan-3-one occurred more frequently in a higher concentration. It was found in the

Anthrosol sampled from Brazil and Germany in concentrations of 46 ng g^{-1} and 19 ng g^{-1}, respectively. In the Brazilian Ferralsol 12 ng g^{-1} were observed. Its concentration in the other soils was always ≤5 ng g^{-1}.

Instead of 5α-pegnan-3β-ol, 5β-pregnan-3α-ol was used as internal standard for the analyses of stanols and Δ^5-sterols in soils and terrestrial sediments in various studies (Bethel et al., 1994; Bull et al., 2001; Evershed et al., 1997; Sweetwood et al., 2009; Van Bergen et al., 1997). This stanol and its corresponding stanone may be better internal standard substances. Further sterols with an ethyl group at C-17 may also serve as internal standards, e.g. pregn-5-en-3β-ol (Bull et al., 2008) obtained by a Wolff-Kishner (Huang-Minlon modification) from pregn-5-en-3β-ol-20-one (Lichtfouse and Albrecht, 1994). Besides sterols with an ethyl group at C-17, isotopically labelled sterols could be used as internal standards. However, the application of other substances than 5α-pegnan-3β-ol and 5α-pregnan-3-one was not tested in the current study.

The amounts of IDCA in the Anthrosols sampled in French Guiana and Germany were 20 ng g^{-1} and 16 ng g^{-1}, respectively. In the other soils the concentration of IDCA was ≤9 ng g^{-1}. This bile acid was proposed by Yamaga et al. (2001) as internal standard for the analysis of bile acids in biological samples. The data presented here showed that it can occur in soils and terrestrial sediments in considerable amounts. For analyses of bile acids in soils and terrestrial sediments 3α,6α,7α-trihydroxy-5β-cholanoic acid was used as IS1 by Bull et al. (2003) and Simpson et al. (1999b). This bile acid can be used only when deposition of pig-derived faeces can be excluded since it is a primary bile acid of pigs (Haslewood, 1967). Isotopically labelled bile acids may serve as an alternative, whereas the number of commercially labelled substances is limited.

4.4.4.4 Precision

The RSDs were in the range of 0.07–0.22 (Tab. 4.3). They were not calculated from repeated measurements of similar subsamples, but from the data of the standard addition experiment. Thus, the TLE of each subsample was spiked with different amounts of analytes covering a large range of concentrations. Furthermore, also the IS1 was spiked in different amounts. Probably, the application of the method to the TLEs of similar subsamples spiked with similar amounts of IS1 would yield lower RSDs as those calculated here.

4.4.4.5 Limits of quantification and detection

The analytes with short retention times (RT) had low LODs and LOQs and the analytes which had longer RT had higher LODs and LOQs (Tab. 4.3). 5β-Cholestanols and LCA had short RT and their LODs were ≤1 ng g^{-1} and LOQs were ≤2 ng g^{-1}. Thus, the method is suitable for the quantification of these biomarkers, which have a high significance for the detection of faeces deposition and can occur in low concentrations (Fig. 4.2a). The LODs and LOQs of 5β-cholestanols found in this study were below the LOD of 50 ng g^{-1} described by Ibanez et al. (2000) for 5β-cholestan-3β-ol and cholest-5-en-3β-ol in soils and are close to the LOQ of 1 ng g^{-1} described by Puglisi et al. (2003) for 5β-cholestan-3β-ol and Δ^5-sterols in soils.

The stanones and the bile acid DCA are also of high significance in the detection of faeces, but had longer retention times and slightly enhanced LODs up to 3 ng g^{-1} and LOQs up to 5 ng g^{-1}. These substances were found in higher amounts in the soils and could be easily quantified in all samples (Fig. 4.2a).

The Δ^5-sterols which were measured with a dilution of 1:5 (Section 4.3.10) and the stanols, and the bile acids which had longer RT showed LODs up to 4 and LOQs up to 10 ng g^{-1}. However, these stanols and Δ^5-sterols were found in high concentrations in soils (Fig. 4.2a) and the LODs of the bile acids were in the range of the LODs of nanograms as summarised by Bull et al. (2002).

4.5 Conclusions

A method was developed for the combined analyses of steroid biomarkers for faeces in TLEs of soils and terrestrial sediments. The method allows the purification of stanols, stanones, Δ^5-sterols and bile acids in order to quantify them by gas chromatography-mass spectrometry. Amounts of faecal biomarkers analysed with saponification of the TLE before the purification and without saponification were compared. This comparison showed that a ‘mild’ saponification of the TLE yielded up to 9 times higher amounts of steroids than analyses without saponification. Rigorous saponification conditions can lead to loss of stanones, but do not cause enhanced liberation of other steroids in comparison to mild saponification conditions. Sequential liquid–liquid extraction allowed extracting the biomarkers from the saponification solution and separating the bile acids from the neutral stanoles, stanones and Δ^5-steroles without acid-catalysed formation of stanones.

Purification of stanols, stanones and Δ^5-sterols was achieved by SPE using 5% deactivated silica gel. Bile acids could be separated from long chain fatty acids by SPE using activated silica gel after methylation with dry HCl in methanol. Stanols and Δ^5-sterols were silylated with HMDS, TMCS and pyridine avoiding a silylation of the keto groups of the stanones in their enol-form. For the silylation of the bile acids BSTFA containing TSIM was used. The method evaluation by standard addition to soil samples showed that stanols, stanones, Δ^5-sterols and bile acids can be precisely quantified with mean recoveries of individual substances ≥85%. The recoveries of biomarkers within each biomarker group did not differ significantly. Thus the method is suitable for quantification of steroid patterns in samples from soils and terrestrial sediments with a wide range of different physico-chemical properties. Samples from archaeological soils were included in the sample set for method evaluation. Data of these samples showed that the method can be used to determine steroids in an archaeological context.

Chapter 5

Ecological engineers ahead of their time:
The functioning of pre-Columbian raised-field agriculture and its potential contributions to sustainability today

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5.1 Abstract

The need to reconcile food production, ecosystem services and biodiversity conservation has spurred the search for more sustainable ways of farming. Archaeology offers examples of prehistoric pathways to agricultural intensification that could be rich sources of inspiration for applying ecological engineering in agriculture today. We examine one set of techniques, pre-Columbian raised-field agriculture in wetlands of Mesoamerica and South America. We point to gaps in knowledge at three levels. First, raised-field agriculture was conducted in a wide range of soils and climates. How different systems functioned was likely to have been correspondingly diverse, but this variation is under-appreciated. At the scale of single farms, nutrient dynamics in raised-field systems likely included complexities quite unusual in 'modern' agriculture, owing to the mixture of aerobic and waterlogged compartments, but data are scarce. Second, at the landscape level there is disagreement about whether fallow periods were necessary, and their eventual roles are poorly understood. Current evidence suggests that self-organizing processes in fallows may have increased the sustainability of some raised-field farming systems in unusual ways. Third, the labor-intensive nature of raised-field farming is held to limit its pertinence to today's global problems, but its real labor costs are unknown. Furthermore, achieving sustainable intensive agriculture will require compensating farmers for ecosystem services they provide. Under a socioeconomic regime that does this, raised-field agriculture could have considerable practical application.

5.2 Introduction

5.2.1 The search for sustainable agriculture

Over the past 10,000 years, humans have turned an ever-increasing part of the biosphere to food production. Human inventiveness in domesticating plants and animals, transforming environments and intensifying agriculture has appeared to allow boundless increases in production. The Green Revolution, beginning in the 1960's, led to the most recent and most dramatic increases in agricultural production (e.g., Evenson and Gollin, 2003). However, by replacing internal controls on ecological processes such as nutrient delivery and pest suppression with external controls such as fertilizers and pesticides, intensive industrial farming, including the Green Revolution, divorced agriculture from ecology (Robertson and Swinton, 2005; Vandermeer, 2011). It is now well recognized that the Green Revolution brought substantial environmental and social costs (Altieri, 2008; Griffon, 2002; Robertson and Swinton, 2005). This model of agriculture has degraded ecosystem services upon which life depends, including water, carbon and nutrient cycles, and climate regulation (Robertson and Swinton, 2005; Perfecto and Vandermeer, 2008). It has favored large-scale cultivators but forced many small-scale cultivators out of farming, helping drive an exodus to cities and an increase in the number of urban poor (Spencer, 2000). Practicing agriculture as ecological and social 'sacrifice' is no longer tenable (Scherr and McNeely, 2008). The challenge of sustainable agriculture is to meet the food and fiber demands of a growing global population while at the same time assuring ecosystem services (Hobbs et al., 2008).

5.2.2 What science is needed?

The fraction of the earth's land surface under agriculture is huge and increasing (Robertson and Swinton, 2005). Managing this land in ways that maintain ecosystem services will require innovation in agriculture and in agricultural policy (Pretty, 2008). The present research and development climate, however, emphasizes the genetic modification of crop plants, with much less attention and resources directed to agroecological innovation (Vanloqueren and Baret, 2009). There is an urgent need to apply ecology to adapting, designing and managing agricultural landscapes that generate benefits for production, biodiversity and local people (Scherr and McNeely, 2008; Vandermeer, 2011). This will require advances in ecology (Robertson and Swinton, 2005), because very few agricultural systems are well understood at the ecosystem level. One ecosystem service (crop yield) has been emphasized to the detriment of others (e.g., the maintenance of biogeochemical cycles) and many ecological interactions important to the functioning of agroecosystems have garnered much less attention than they deserve (Gliessman, 2007; Vandermeer, 2011), particularly in the context of low-input agriculture (Drinkwater and Snapp, 2007). Research is needed to identify the organisms and ecological processes that play key roles in the functioning of agroecosystems (Robertson and Swinton, 2005) and ways must be found to explain and promote the value of the services that ecosystems provide for humans.

5.2.3 Folk knowledge, contemporary and past, as a source of inspiration for agroecological innovation

Successful agroecological solutions are likely to be strongly case-specific (Doolittle et al., 2002; Pretty, 2008). The folk knowledge of smallholder farmers is one source of inspiration for the diverse solutions that will be needed (Denevan, 1995; Altieri, 2008; Martin et al., 2010; Vandermeer, 2011). Often forced to find ways to produce food in unfavorable environments, these farmers have developed ingenious adaptations that do not depend on costly external inputs and that allow long-term use of limited resources such as land, nutrients or water (e.g., Mollard and Walter, 2008). Because "traditional" agriculture is often considered to be of low productive potential, the knowledge of smallholder farmers is usually ascribed little relevance to solving today's problems (Erickson, 1992). However, productive potential of such systems has often been underestimated (Vandermeer, 2011). Furthermore, when comparisons are focused not solely on the production of a marketable commodity but on metrics that integrate total production, ecosystem services and positive effects on biodiversity, traditional agriculture wins hands down over modern industrial agriculture (Altieri, 2008).

A subset of this knowledge that has attracted particular attention is the "fossil" folk knowledge of past cultures. Long before the origin of Western-style industrial agriculture, many other cultures engaged in agricultural intensification, sometimes employing techniques that have virtually disappeared today. History and prehistory offer many examples of diverse trajectories to intensification (Denevan, 1995; Erickson, 1992; Guttmann-Bond, 2010; Thurston and Fisher, 2006). By the time depth they offer and the multiplicity of cases permitting a comparative approach, archaeological studies can provide unique insights into the sustainability of agricultural systems, the sources of their resilience, and their vulnerability, both to extrinsic environmental factors such as climate change (Kemp et al., 2006; Branch et al., 2007) and to factors intrinsic to societies (Janssen and Scheffer, 2004). The data suggest that some of these prehistoric intensive agricultural systems were sustainable for centuries (Armillas, 1971; Coe, 1964; Denevan, 1995; Erickson, 1992), some having been used apparently continuously for over 1000 years (Gliessman, 1991; Mathewson, 1987). It would be fanciful to suppose that any of these agricultural systems are panaceas – after all, many have disappeared, and it is obviously important to know the reasons why – or that they could be transposed intact into today's very different social, techno-economic and environmental contexts (e.g., Siemens, 2000; Lombardo et al., 2011). Could the "fossil" local knowledge of extinct cultures, as inferred from archaeological, geo-archaeological and archaeo-botanical studies, and from the results of rehabilitation experiments, provide insights useful for devising sustainable agricultural intensification in the 21st century?

Archaeological studies of pre-Columbian Latin America have provided two examples of once widely practiced, but now virtually extinct, agricultural techniques that are considered to have real potential for contributing to the design of sustainable agro-ecosystems today (Darch, 1988; Doolittle et al., 2002; Lehmann et al., 2006; Siemens, 2000). The techniques employed appear not only to have provided novel solutions to strong environmental constraints on food production, but also had effects on ecosystem functioning that 21st century humans would consider positive.

The first of these examples is the discovery in forested parts of Amazonia of soils that were amended, intentionally or unintentionally, with large amounts of charcoal (and with nutrient-containing organic matter and ash) by pre-Columbian farmers (Glaser, 2007; Glaser and Birk, 2012; Glaser et al., 2001b). Their biochemical properties make these *terra preta* and *terra mulata* soils much more fertile than the Oxisols and Ultisols from which they are derived (Glaser, 2007; Glaser et al., 2001). Investigation of these soils by archaeologists, soil scientists and ecologists has spurred burgeoning interest in biochar (Atkinson etal., 2010; Glaser, 2007; Glaser et al., 2002; Lehmann, 2009; Robertsetal., 2010). In addition to conferring greater fertility, biochar amendment is attracting interest as a way of storing carbon durably in soils (Glaser, 2007; Glaser et al., 2002; Lehmann et al., 2006), thereby removing carbondioxide from the atmosphere. Although the underlying mechanisms are unclear, effects of biochar on soil biogeochemical processes may also lead to reduced emission from soils of other greenhouse gases, such as nitrous oxide (Yanai et al., 2007). Experimental studies of "*terra preta nova*", inspired by archaeological and geo-archaeological studies, are showing promising results (Steiner et al., 2007, 2008). Furthermore, forest on and near abandoned pre-Columbian anthropogenic soils is richer in tree species (Clement and Junqueira, 2010) and in agrobiodiversity (Clement et al., 2004; Junqueira et al., 2010; Major et al., 2005) than that growing on nearby unmodified soils, demonstrating the resilience of ecosystems to even several centuries of intense human occupation.

The second example of pre-Columbian agricultural techniques that have spurred interest in potential applications today is the diversity of forms of wetland agriculture (see Denevan et al., 1987; Valdez, 2006; Sluyter, 1994; Denevan, 2001). During the late Holocene many seasonally flooded tropical savannas (and some lakeshore habitats) of South America and Meso-America were transformed into vast agricultural landscapes through the construction of raised fields by Native Americans. Raised-field agriculture provided pre-Columbian farmers with better drainage, soil aeration, moisture retention during the dry season and increased fertility, as well as possibly easier weeding and harvest. In addition, in some areas channels between raised fields were used for fish and turtle farming and as a renewable source of nutrients for the soil. Estimates in the Beni savannas of the Bolivian Amazon (roughly the size of the UK) suggest that in this region alone up to 1 million ha of ancient raised earth platforms were constructed by Pre-Columbian cultures beginning around 400 BC (Erickson, 2006; Saavedra, 2009). The extent of these constructions, which must have developed over a long period, suggests that this was a form of intensive agriculture that probably supported dense populations in the basin of a very active river system over several centuries. Ironically, while today about 90% of local communities in this region are impoverished, much of this ancient agricultural landscape lies abandoned or under-utilized. The contrast between the past, with high population densities supported by sustainable agriculture, and the present, with an impoverished, thinly scattered human population, is striking.

Nordenskiöld (2009 [1916]) was the first 20th century explorer to remark upon raised fields, but they attracted no broad interest until much later. Geographers and archaeologists have studied the ecology and use of wetlands in the pre-Hispanic Americas since the 1960’s (Denevan, 1966, 1970, 2001, 2006; Luzzader-Beach and Beach, 2006). The systems that Native American farmers developed include the famous *chinampas* of the Basin of Mexico (e.g., Sanders et al., 1979). Some form of wetland agriculture, culminating in *chinampas*, has been continuously practiced in these environments for perhaps 2000 years (Armillas, 1971; Coe, 1964). Various other raised-field systems were developed in the altiplano of Bolivia and Peru (Erickson, 1992, 2003; Kolata, 1996), in marshes in inter-Andean valleys (Bray et al., 1987; Knapp and Mothes, 1999; Wilson et al., 2002), and in seasonally flooded savannas in the lowlands of Mesoamerica and South America. Such savannahs occupy huge expanses of South America, in the Llanos de Mojos of Bolivia (Denevan, 1966; Erickson, 1995; Saavedra, 2009; Walker, 2004), in the Llanos of Venezuela (Zucchi and Denevan, 1972; Denevan and Zucchi, 1978; Spencer et al., 1994) and Colombia (Reichel-Dolmatoff and Reichel-Dolmatoff, 1974), and in the Mompos Depression in Colombia (Plazas and Falchetti, 1990). Smaller areas are present in the Mexican states of Veracruz (Siemens et al., 1988) and Tabasco (Gliessman, 1991), in the Maya lowlands of Mexico and Belize (Gliessman et al., 1985; Sluyter, 1994), in the coastal savannas of the Guianas (Rostain, 1994, 2008a), in the Guayas basin of southeastern Ecuador (Stemper, 1987) and even in southern regions of Chile (Dillehay et al., 2007). Characterized by annual cycles including rainy-season flooding, dry-season drought, and often widespread fires, many of these areas are thinly inhabited today, as noted above, and some are considered suitable only for extensive cattle grazing. Raised-field agriculture was conducted in a great range of environments, from the highland plateaux of the Andes (Erickson, 1992; Kolata, 1996) and inter-Andean valleys (Bray et al., 1987; Wilson et al., 2002) to the hot lowlands, from the karstic Yucatan peninsula (Dunning et al., 2002) to the acid soils of the Llanos de Mojos (Boixadera et al., 2003; Hanagarth, 1993). Yet raised-field agriculture had virtually disappeared by the colonial period, with only scattered anecdotal descriptions of its techniques by a few early chroniclers (De Las Casas, 1986 [1560]; Gumilla, 1963 [1791]).

Understanding the reasons for the abandonment of raised-field agriculture in different regions of the Americas requires more detailed archaeological and paleoecological research in each particular region. However, in several regions the impact of post-Columbian diseases on agricultural populations may have played a major role in the demise of raised-field agriculture. These epidemics and pandemics were arguably the most rapid, thorough, and widespread to have occurred during the late Holocene (Crosby, 1972; Lovell, 1992). According to the estimates of some authors, they may have resulted in the loss of as much as 80-95% of the agricultural population across the Neotropics (Dobyns, 1966; Lovell and Lutz, 1995). Labor-demanding raised-field agriculture must have been significantly impacted by such substantial reductions of the labor force. In other cases, however (for example, Hertenrits in coastal Suriname [Versteeg, 2008]), raised-field agriculture was abandoned several centuries earlier. In most sites, the timing and the cause(s) of abandonment of raised-field farming are still unknown. Apart from the *chinampas*, with only few exceptions raised fields are no longer used today by Amerindians (Denevan and Schwerin, 1978; Grenand, 1981; Crews and Gliessman, 1991).

5.2.4 Advantages of raised-field agriculture and its pertinence to today's problems

How pre-Columbian farmers managed to cultivate seemingly inhospitable wetland environments, sometimes for centuries, has long fascinated archaeologists, geographers and agronomists (Denevan, 1970; Lambert et al., 1984; Morris, 2004). Raised-field agriculture has been suggested to have several positive effects on food production. Nutrients were concentrated on cultivated surfaces whose topsoil was made deeper with amendments. Water was managed to avoid flooding and sometimes to promote irrigation;

embankments were sometimes constructed around groups of raised fields to protect them from river overflow (Erickson and Walker, 2009). The wetland component of the system is postulated to have played roles in supplying nutrients to crops and sometimes food for people (Erickson, 1992, 2000; Kolata, 1996). Finally, crops were in some instances provided with more favorable micro-climates (greater frost tolerance in cold highland environments [Erickson, 1992; Kolata and Ortloff, 1989]). Researchers have also highlighted the apparent sustainability of raised-field agriculture (Crews and Gliessman, 1991; Denevan, 1995).

This interest in raised-field agriculture has led to several experimental studies aimed at understanding how it worked and testing its proposed benefits (Gliessman, 1991; Erickson, 1995; Kolata, 1996; Barba et al., 2003; Morris, 2004; Saavedra, 2009), or even attempting to rehabilitate it as a viable system of food production (Muse and Quinteros, 1987; Morris, 2004). These studies have often demonstrated high yields per unit land cultivated (Sanders et al., 1979; Arce, 1993; Muse and Quinteros, 1987; Saavedra, 2009) and have shown how raised-field agriculture can enhance ecosystem services such as nutrient retention in wetlands (Kolata and Ortloff, 1989; Biesboer et al., 1999; Carney et al., 1996), a topic of great importance today. Despite these demonstrated effects, however, there is still controversy about the relevance of raised-field farming to agriculture in the 21st century. Although the productivity of raised-field agriculture in terms of yield per unit land cultivated is demonstrated, the most extensive projects to rehabilitate these systems are considered by their critics to have failed (Bandy, 2005; Chapin, 1988). Meanwhile, proponents of many of these projects remain optimistic about their potential (Denevan, 2001; Gliessman, 1991; Saavedra, 2009), and new projects are underway (Saavedra, 2009).

Why is there such uncertainty in interpreting the prospects for rehabilitating raised-field agriculture for food production today? We believe there have been two major problems. The first is a lack of insight into how raised-field systems functioned in terms of their agroecology. This problem can be broken down into two sub-problems. (i) A failure to appreciate the diversity of ecological situations in which wetland agriculture was conducted, and the corresponding diversity of adaptations and constraints, has led to overgeneralization and sometimes to transposition of historical models into inappropriate present-day contexts (Chapin, 1988; Lombardo et al., 2011). (ii) A failure to look beyond the Neotropics has blinded workers to the existence of present-day analogues of these systems that are demonstrably not only productive but also economically viable, and that could help provide realistic guidelines for attempts to reconstruct raised-field agriculture in the Americas. The second major problem is that the researchers and farmers involved in these experiments have had to confront sociocultural and economic contexts over which they have little control, and which have often been highly unfavourable to the success of the experiments. Critics have emphasized what they consider a lack of congruence between raised-field farming techniques and today's social, cultural and economic conditions, or even a failure on the part of the experimenters to take these conditions into account, but the reality appears to be more complex. For example, Chapin (1988) notes that one Mexican attempt to establish raised-field agriculture as a food-production system ultimately failed not because of agroecological constraints (although these were formidable) but because no provisions were made to market the harvest, which rotted or was sold at give-away prices. He did not mention, however, that the experiment in question was a state-run, large-scale, poorly planned top-down enterprise that tried to imitate a smaller experiment designed by agroecologists and conducted with more effective farmer participation. According to its designers, this smaller project was producing promising results before being interrupted by reduction of its funding when the large-scale projects began (Gliessman, 1991). Experiments faced with such problems can hardly be expected to yield conclusive results on the feasibility of raised-field agriculture today. Similar problems are faced by any move to adopt ecological agriculture in today's world, where both local knowledge and rural societies have been weakened or destroyed by the hegemony of the industrial agricultural model (Vandermeer, 2011).

Rebuilding knowledge requires identifying gaps in our understanding. We identify several major gaps in our understanding of raised-field agroecosystems, by analyzing their functioning at three different levels.We first examine their ecological functioning at the scale of the local plot: How did humans engineer environments by constructing and maintaining raised fields and the wetland matrix surrounding them, and what were the effects of this engineering on ecological interactions in active wetland farms? Second, we examine the system's functioning at a larger scale of space and time, that of the landscape over numerous cultivation cycles. Were fallows required? If they were, how did human engineering activities affect ecological interactions in fallows, and how did the integrated field/fallow cycle function? Whether raised-field farming can actually contribute to solving human problems depends on combining these "hard agroecology" approaches with "soft agroecology", taking into account the sociocultural and economic context in which farming activities are embedded (Dalgaard et al., 2003). Thus, in the third and final part of this essay, we ask whether raised-field agriculture is adapted to today's social and techno-economic context. In this third part we focus on the one aspect that is most frequently cited as a factor likely to limit the pertinence of raised-field agriculture today, its labor-intensive nature.

5.3 Towards a systems-level understanding of raised-field agriculture

5.3.1 Raised-field agriculture as ecological engineering

Wetland agriculture offers great scope for engineering, because slight variations in elevation dramatically affect drainage, which in turn affects the availability of water and oxygen, and thereby all the biological, chemical and physical processes that determine nutrient availability (Moser et al., 2009). Moving relatively small amounts of earth can thus create a mosaic of microenvironments differing in their ecological functioning. Farming systems can exploit this heterogeneity in diverse ways. Furthermore, once created, the heterogeneity produced by human earthmoving activities is likely to have long-lasting ecological effects.

5.3.1.1 Morphological diversity of raised fields and its functional significance

Raised fields exhibit great morphological diversity among (e.g., Denevan, 2001), and even within sites (e.g., McKey et al., 2010). However, their initial height appears everywhere to have been fairly similar. Although mounds in many sites have been partially eroded, both archaeological data (e.g., profiles of raised fields buried by ash from the eruption of Quilotoa in Ecuador [Knapp and Mothes, 1999; Wilson et al., 2002]) and contemporary analogues (Vasey et al., 1984) show that planting surfaces were usually elevated about 0.5–1 m above the mean rainy-season water level of the floodplain or swamp. However, some raised fields are up to 2 m high (Darch, 1988) or even up to 3 m (Vasey et al., 1984). Such variation could be related to local expectation of extreme flood events, but no information exists to evaluate this hypothesis. Platforms varied in size, shape and orientation. Typical shapes range from linear and curvilinear ridges to square, rectangular or inter-locking platforms, but round, rectangular and multi-sided fields are also known (Darch, 1988; Denevan, 1970; McKey et al., 2010). Raised fields are often grouped in parallel series to form ladder- and checkerboard-like arrangements, which are sometimes bordered by ditches or embankments. Size of individual fields also varies enormously. Ridged fields may be up to 25 m wide and 100 m long or longer and some round mounds are only 1–1.5 m in diameter. Organization and orientation of fields appear engineered to suit local hydrological conditions (Darch, 1988). For example, ridges are oriented parallel to slopes to facilitate drainage and perpendicular to slopes to enhance water retention (McKey et al., 2010). Similarly, in some areas of the Beni, raised fields were built in flat areas (such as old lake beach ridges or levees) and ditched fields, promoting drainage, on gentle slopes (Lombardo et al., 2011). In some cases, raised fields are combined with dykes, sluices or levees that gave some control over the general water level (Darch, 1988). Canals of varying widths, permanently or seasonally filled with water, were created between the platforms. Depth of canals also varied. Around Lake Titicaca, most canals were at least 1 m below the present surface and some were up to 2 m deep (Erickson, 1992). In other sites, canals were absent, or almost so, and the spaces between raised fields constituted a shallow, usually only seasonally flooded, matrix (McKey et al., 2010).

5.3.1.2 Sources of information about how raised-field agroecosystems functioned

Whereas the morphology of pre-Columbian raised-field landscapes has been extensively described, few studies provide actual data on their formation, chronology and use: “even for the *chinampas* of the Basin of Mexico, only four studies actually present primary data recovered through scientific investigation data recovered through scientific investigation” (Beach et al., 2009). Twenty-five years ago, Vasey et al. (1984) lamented that while the morphology of raised fields had been well described, very little work had been devoted to the ecological functioning of raised-field systems. Despite the progress reviewed here, large gaps remain. Data on processes underlying the productive capacity of raised-field systems, and the environmental services they are supposed to have ensured, are scarce and fragmentary. What we know, or can surmise, about how raised-field farming functioned in the past comes from three sources of information. First, archaeologists, geographers, geoarchaeologists and archaeobotanists have provided some data on how these systems were constructed (e.g., Kolata, 1993; McKey et al., 2010), how their soils were managed (Wilson et al., 2002), and what crops were grown on them (e.g., Iriarte et al., 2010; McKey et al., 2010; Pearsall, 1987; Siemens et al., 1988; Turner and Miksicek, 1984). Second, archaeologists, working with ecologists, agronomists, soil scientists and local farmers, have conducted experiments aimed at replicating raised-field farming to study its workings and in some cases to rehabilitate it as a viable method of food production (Erickson, 1992; Erickson and Candler, 1989; Gliessman, 1991; Saavedra, 2009). Third, geographers and agronomists have reported data on contemporary farming systems that are in at least some respects modern analogues of ancient raised-field systems. Apart from the *chinampas*, which persist today in much altered form near Mexico City (Torres-Lima et al., 1994), and less altered systems similar to *chinampas* in Tlaxcala, Mexico (Crews and Gliessman, 1991), the closest analogues are found in southern China (Kleinhenz, 1997; Luoand Lin, 1991), SE Asia (IIRR, 1990) and Oceania (Kirch, 1978). With the exception of a few early papers (e.g., Denevan and Turner, 1974; Vasey et al., 1984), the literature on these Old-World systems appears to have been completely ignored by those working on Neotropical raised-fields and the prospects for their re-establishment.

Each of these data sources presents strong limitations. Whereas artifacts and ecofacts studied by modern archaeological methods do reveal a wealth of information, one always wishes for greater resolution and richer detail. Experimental studies can provide these, but there is always the possibility that environments, techniques, labor organization, plant material or all of these differ in important ways from those that existed centuries before when fields were being used (Gondard, 2006; Lombardo et al., 2011). Furthermore, some experiments in re-establishment have suffered from methodological shortcomings that limit their pertinence as tests of the feasibility of raised-field agriculture today (Lombardo et al., 2011). Finally, no contemporary systems are exact analogues of prehistoric systems. Agricultural systems in Asia and Oceania that combine cultivation of upland crops on raised fields and of wetland crops (usually rice or taro) in ditches offer the closest parallels. Pre-Columbian raised-field farmers in the Neotropics appear to have had no waterlogging-tolerant wetland crops similar to rice or taro. Like other “orphan systems” of marginal environments (Mollard and Walter, 2008), raised-field systems of Asia and Oceania, and some similar systems in Africa (e.g., rice cultivation in reclaimed mangrove areas by the Diola of Senegal, Pélissier, 2008 [1966]), have been comparatively neglected by researchers, but the literature that does exist suggests they confer distinct advantages (IIRR, 1990; Kleinhenz, 1997). Even paddy rice farming systems, part of the life-support system for a large part of the world’s population, were considered poorly known as ecosystems 25 years ago (IRRI, 1985). Roger (1996) has since published a remarkable synthesis of the considerable information that does exist. His treatment, emphasizing ecological interactions and how they are affected by agricultural intensification, suggests numerous questions for research on Neotropical raised-field systems. Some of these are addressed below (see Sections 5.3.2.3.2 and 5.3.2.3.3).

5.3.2 Agroecological functioning at the field level

What factors constrained food production in raised-field systems, and how did farmers manage them? What were the environmental consequences of management, and what mechanisms underlay crop production and other ecosystem services? The answers to these questions are probably as diverse as the environments in which raised fields were constructed. These environments range from sea level to over 4000 m elevation, covering a great range of soils and climates. Some are in seasonally flooded basins, others in permanently wet habitats. Three key factors managed by farmers to ensure production in raised-field systems have been identified, but their relative importance and the underlying mechanisms are likely to be highly variable among different systems.

5.3.2.1 Frost reduction

This first factor could have been important only in the Altiplano. Tropical montane habitats undergo extreme temperature fluctuations between day and night. At high elevations, nocturnal frosts may kill crops, even Andean species such as potatoes. Owing to its high thermal capacity, water acts as a temperature buffer, re-radiating during the night heat energy stored during the day. Proximity of fields to bodies of water, such as the canals between raised fields, could thus protect crops against frosts. Knapp (1988) showed that minimum night-time air temperatures above canals and above raised fields around Lake Titicaca were 1–2 °C higher than above areas without raised fields at similar elevation nearby. The magnitude of this effect should increase with increasing ratio of water to land in the zone. This may explain why ridge fields were narrow and canals were broad in this system (Gondard, 2006).

5.3.2.2 Water management

Farmers constructed raised fields to create drained platforms for planting crops, but their earthmoving activities often had other additional purposes and consequences. In some regions, raised fields were associated with canals, causeways (Erickson and Walker, 2009), reservoirs, habitation mounds and structures interpreted as fish weirs (Erickson, 2000). Water management thus played multiple roles in the strategies of some of these peoples. Fish and other aquatic organisms are believed to have been important protein sources in some areas (Darch, 1988; Erickson, 2000), as they are in some Asian paddy rice and mixed upland/wetland high-bed systems today (Guo and Bradshaw, 1993; Luo and Lin, 1991). Despite the elaborate morphology of pre-Columbian earthworks in some sites, it is important to note that most were dependent on rainwater, groundwater, or lakeshore habitats, with only limited contribution of river water (Morris, 2004). There was thus in many systems little need – or opportunity – for top-down control of water flow and allocation; each group of fields could thus be managed largely independently of others (Erickson, 2003).

In addition to drainage, management also aimed at conserving water for long- and short-term droughts, using the stored water to extend growing seasons (Erickson, 1992). In some sites, ditches and canals were deep enough to have exposed groundwater through at least part of the dry season (Erickson, 1992), and hand irrigation (splash or bucket), as practiced today in the *chinampas* and other modern analogues (Crews and Gliessman, 1991; Vasey et al., 1984), enabled cultivation into the dry season (Erickson, 1992). Work in experimental raised fields has demonstrated the advantages such water management could confer. Experimental raised fields near Lake Titicaca gave some yield during the severe 1982–1983 El Niño drought, when other types of farms nearby yielded nothing, and gave excellent yields in 1985–1986, when flooding devastated other types of farms (Erickson, 1992). Extending the growing season may have been particularly important in raised-field sites located in hyperseasonal savannas, such as those in the Llanos of Bolivia and Venezuela and the coastal savannas of the Guianas. Seasonally fluctuating water levels must have conditioned agricultural cycles, with water shortage precluding farming in the dry season. As water levels receded, raised fields could have increased water availability to crops by two mechanisms. First, high organic matter content in mound soils, which would be expected under continuous mulching (see next section), could maintain greater moisture availability. Second, mound soils could receive water by capillary movement ("wicking action") from adjacent canals or the flooded basin (Kleinhenz et al., 1996b). It has also been speculated that groundwater could rise by capillary movement to infiltrate the root zone of elevated planting surfaces from beneath (Erickson, 1992). This phenomenon is exploited by engineers in "subirrigation" systems (Allred et al., 2003). Although it has frequently been considered important in Neotropical wetland agriculture, only one study has attempted to study this process in any detail. Crossley (2004), working in modern-day *chinampas*, found little support for an important role of subirrigation processes and noted "the tremendous complexity of the soil and plant properties required for subirrigation to occur naturally, and function significantly". However, capillary rise is known to be an important factor affecting water availability to plants in a wide range of cultivated (Ayars et al., 2006; Nosetto et al., 2009) and natural ecosystems (Döll et al., 2003; Rodriguez-Iturbe et al., 2007) with shallow water tables, and we would find it surprising were it not to play an important role in raised-field agroecosystems. This topic certainly deserves deeper study.

Studies examining the hydraulic properties of soils of contemporary high-bed systems in Asia suggest functional links between hydrology and the optimal design of raised-field systems. Raised beds facilitate drainage when water level is high (by allowing horizontal removal of excessive soil water when vertical flow is negligible) and furrows increase the supply of water to plants on beds (by “wicking action”, i.e., capillary flow) when conditions become dry. Both effects are weaker in the center of beds, which are quickly saturated in the rainy season and quickly dry out in the dry season (Kleinhenz et al., 1996b). This helps explain why yield decreases from the edge to the center in some raised-field systems (Kleinhenz et al., 1996a). These processes should affect the optimal dimensions of raised fields. If space allocation to raised fields is to be maximized, they should be as wide as practical. However, if soils are inundated for long periods by continuous rainfall and if soil water is deficient during periods of limited rainfall, narrower fields are called for. Both effects of raised fields (drainage and wicking) would be maximized in small, round raised fields, suggesting that this form would be optimal where seasonal extremes in flooding and drought are most marked. Data on flooding regimes within and among different regions of pre-Columbian raised-field sites are not of sufficient precision to test for the suggested correlation. However, the Llanos de Mojos, where large seasonal variations in water level are known to occur, are not characterized by small round raised fields; long, broad ridges appear to be much more frequent.

Perusal of the literature on wetland ecology leads to numerous other questions about hydrological aspects of raised-field construction and management. Raised-field construction increased the soil surface roughness of previously flatter landscapes. Did this increase the depression storage capacity of the affected areas (Darboux et al., 2002)? Many of the basins in which raised fields were constructed show great seasonal variation in water levels. Did farmers cultivate different parts of the landscape at different seasons? It has usually been assumed that only the raised surfaces were cultivated (but see Parsons and Shlemon, 1987). Observations by Gliessman (1992) among contemporary indigenous farmers in Tabasco, Mexico, call this assumption into question. He described a system in which maize is planted on higher ground around flood-prone areas during the wet season, then on lower ground as water levels drop during the dry season. Pre-Columbian raised-field farmers could have similarly exploited the topographic heterogeneity they created to extend cropping seasons in raised-field agriculture. Present-day Asian raised-field systems such as the sorjan cropping system in Indonesia (Domingo and Hagerman, 1982;

IIRR, 1990) and permanent-high-bed systems in southern China (Kleinhenz, 1997; Luo and Lin, 1991) exploit such heterogeneity by growing upland crops, usually vegetables, on raised fields during the rainy season and wetland rice in the surrounding furrows or canals.

Considering the importance of hydrology in raised-field systems, the absence of detailed treatments (and particularly of surface water–groundwater interactions) constitutes a major gap in efforts to understand the functioning of these systems and to apply this knowledge in agriculture today.

5.3.2.3 Nutrient management

Raised-field farmers added nutrient-containing materials to the elevated planting surfaces they created. Observations of modern analogues, and in some cases inferences from archaeological data, give indications of the kinds of material that were added to raised fields as nutrient sources. However, little work has examined the dynamics of soil–crop–nutrient relations in any detail. Vasey et al. (1984) noted this over 25 years ago and it is still largely true today. In raised-field environments, well-aerated and waterlogged soil compartments are both present, and vary in distribution in space and time. Aquatic and terrestrial environments, and their ecotones, are all part of the whole system. These peculiarities should introduce complexity in nutrient dynamics in raised-field agriculture, but we know very little about this aspect of their functioning. We will first review what is known about the kinds of nutrient-containing amendments added to raised fields and then explore the processes that may underly soil–crop–nutrient dynamics.

5.3.2.3.1 Different types of materials added to raised fields

Materials added to raised fields likely came from several sources. Some may have been imported from outside the raised-field system. Kitchen waste, fish remains, ash and charcoal from hearth fires are potential sources, although these were more likely to be important in home gardens than in extensive raised-field systems relatively distant from habitation sites. Farm animal manure is used in present-day *chinampas*, and formerly human excrement was, too (Coe, 1964; Armillas, 1971). Wilson et al. (2002) interpreted some soil micro-morphological features as the possible result of animal manure application in raised fields in Ecuador. Erickson (1994) noted the presence of abundant carbonized seeds of wild plants in ancient raised-field soils near Lake Titicaca. He postulated that these could have been derived from burnt llama dung, which these people may have used for cooking. However, as noted by Gondard (2006), the carbonized plant material could also have been produced by the burning of weeds gathered from agricultural fields, or by the burning of fallow vegetation (see Section 5.3.2.4). Finally, in one site in French Guiana, farmers appear to have strip-mined topsoil from adjacent areas and deposited it onto raised fields (McKey et al., 2010). Raised fields in Caño Ventosidad in the Llanos Occidentales of Venezuela were also constructed with material brought in from elsewhere, not from the canals near these raised surfaces (Gondard, 2006). Other materials were derived from recycling within the raised-field system. Crop residues, for example, are virtually universally incorporated into fields in analogous contemporary systems (Crews and Gliessman, 1991; Denevan and Turner, 1974; Domingo and Hagerman, 1982; IIRR, 1990; Kirch, 1978; Kleinhenz, 1997), and there is some evidence for incorporation of maize leaf residues into pre-Columbian raised fields in French Guiana (McKey et al., 2010; Iriarte et al., 2010). Addition of crop residues as mulch confers the added advantage of suppressing weeds (Boucher et al., 1983; IIRR, 1990; Kirch, 1978).

In some systems, the most important source of nutrient-providing material added to raised fields may have been the organic matter produced and accumulated in the aquatic component of these systems. Transferring vegetation and bottom sediments to the cropping area is an inevitable consequence of maintaining canals, and such transfer is universal in extant analogous systems (Crews and Gliessman, 1991; Denevan, 2001; Vasey et al., 1984). In present-day *chinampas*, organic matter-rich sediments accumulate in the wetland part of the system, particularly during each rainy season, and this muck is added to fields (Darch, 1988). This muck includes crop residues and other material lost in runoff from raised fields and material derived from aquatic plants. Macrophytes and algae were abundant in canals of experimental raised fields at Tiwanaku (Carney et al., 1993) and water hyacinth (*Eichhornia crassipes*) is abundant in the seasonally flooded savannas of the Llanos de Mojos (Gondard, 2006; Lee, 1997). Various macrophytes are abundant in the contemporary *chinampas*-like system in Tlaxcala, Mexico, and contribute to formation of mulch added to raised fields (Crews and Gliessman, 1991). The top 70 cm of cores from ancient raised fields in Pulltrouser Swamp include significant amounts of water lily (*Nymphaea sp.*) pollen, substantiating the manuring hypothesis (Wiseman, 1983 in Darch, 1988). Macroalgae, microalgae and aquatic and amphibious plants produce litter with much lower C:N and C:P ratios that is decomposed more rapidly than that produced by other plants (Enriquez et al., 1993). Both algae and aquatic macrophytes exhibit “luxury consumption” of N and P, assimilating these nutrients in excess of their needs and storing them for use under nutrient-deficient conditions (Roger, 1996). Finally, the aquatic compartment of raised-field systems may contain nitrogen-fixing bacteria and cyanobacteria. In experimental raised fields around Lake Titicaca, nitrogen fixation by cyanobacteria in the flooded canals furnished nitrogen- and mineral-rich detritus that could be added to fields (Biesboer et al., 1999). The aquatic fern *Azolla*, associated with symbiotic nitrogen-fixing cyanobacteria (*Anabaena*), is frequent in slack-water canals of some raised fields (Vasey et al., 1984) and in the seasonally flooded savannas of the Llanos de Mojos (Gondard, 2006). The importance of the *Azolla/ Anabaena* symbiosis to the nitrogen economy of paddy rice systems has been demonstrated (IRRI, 1985; Roger, 1996), and it appears likely to have played a significant role in some raised-field systems as well (Biesboer et al., 1999). Another nitrogen-fixing symbiosis, the actinorhizal association between *Frankia* and the riverine tree *Alnus*, is important in *chinampas* and in the similar system in Tlaxcala. *Alnus* trees, frequently planted near the *chinampas* platforms, supply nitrogen-rich litter (Crews and Gliessman, 1991). Litter from leguminous crops planted on platforms could also enhance nitrogen availability (Crews and Gliessman, 1991).

5.3.2.3.2 Importance of nutrients from aquatic resources

However, the importance of aquatic resources as a nutrient source is likely to have varied widely among systems, depending on at least four factors. First, the potential was probably much greater in permanently flooded marsh and lakeshore habitats than in seasonally flooded wetlands (Lombardo et al., 2011). Fish, turtles, macroalgae and aquatic macrophytes can persist only if the basin (or at least some canals) is filled with standing water year-round. With only seasonal flooding, aquatic life is much more limited. However, seasonally flooded basins do support microalgae, as well as flooding-tolerant sedges and grasses, and biomass from these could be added to raised fields. A second factor influencing the potential importance of the wetland component is the ratio of water to land surface. If the area covered by water is large relative to the surfaces of planting platforms, greater nutrient concentration can be achieved (Vasey et al., 1984). Thus, in Altiplano raised fields, narrow platforms and broad canals may have conferred not only a thermal advantage but a fertility advantage as well. A third factor is the presence of uncultivated land near fields that can be “mined” for topsoil to supplement the cultivated surfaces (Vasey et al., 1984). If material can be extracted from a sufficiently large area of uncultivated land around the field complex and brought to cropping areas (see for example McKey et al., 2010), the water-covered area surrounding fields may be irrelevant as a source of nutrients. Finally, the productivity of the aquatic compartment is likely to vary enormously among environments where raised-field agriculture was practiced. Productivity of aquatic systems – and thus their importance as a source of nutrients for raised fields – is likely to be higher in areas with relatively nutrient-rich young volcanic soils (such as those in the Andes, the Valley of Mexico, and Tlaxcala), or soils derived from calcareous sedimentary rocks (e.g., in the Maya lowlands), than in areas with old, highly weathered soils like those of seasonally flooded South American savannas. The importance of nitrogen fixation is likely to show parallel variation. Nitrogen fixation is energetically advantageous only if levels of phosphorus are adequate (Vitousek and Howarth, 1991). Furthermore, nitrogen fixation usually occurs at low rates in acid soils. In some prehistoric raised-field complexes in coastal savannas of French Guiana, the paucity of phosphorus and nitrogen is suggested by the occurrence of two genera of carnivorous plants, *Drosera* and *Utric ularia* (D. Renard et al., unpublished data). Under such oligotrophic conditions, the aquatic compartment may be less important as a source of nutrients for amending raised fields. There may also be considerable variation in fertility within raised-field regions. The white-water (sediment-rich) Mamore River runs from its source in the Andes through the western part of the Beni, its overflow sometimes affecting raised fields, whereas blackwater (sediment-poor) rivers run through the Beni's eastern sector (Lombardo et al., 2011).

Farmers in regions where the waters surrounding raised fields are oligotrophic may have been able to render them more eutrophic. For example, lower soil horizons are less acidic than surface horizons, and deep ditches cut into them could reduce the acidity of water in ditches, facilitating nitrogen fixation if other conditions allow it (Vasey et al., 1984; Gondard, 2006). However, it is uncertain how long this effect would last, because water in ditches would become more acidic as organic matter accumulates in them and bases are leached out of newly exposed soil horizons (Vasey et al., 1984). Another way by which farmers in such areas could render the aquatic compartments of raised-field systems more eutrophic – and thereby enhance their role in recycling nutrients to field surfaces – is to import large quantities of nutrients from outside sources into the basins where raised-field agriculture was conducted.

However, work in paddy-rice systems shows that nitrogen fixation by bacteria and cyanobacteria can also play significant roles even in acid soils (Roger, 1996). Furthermore, both pH and nutrient availability can show great variation with seasonal drying and rewetting cycles (Roger, 1996), which could drive seasonal patterns in nitrogen fixation. These and other complexities likely to characterize nutrient dynamics in raised-field agriculture are treated in the following section.

5.3.2.3.3 Nutrient dynamics in aquatic and terrestrial compartments

The ultimate determinant of plant performance and (in crop plants) yield is not the total amount of nutrients, but their availability. The aerobic or anaerobic status of environments influences the availability of different nutrients in complex fashion (Chepkwony et al., 2001; Kirk, 2004; Vasey et al., 1984). Raised-field systems combine aerobic and anaerobic environments, and material is transferred between the two. All of these facts should lead to very complex soil–crop–nutrient dynamics, but this aspect of the ecology of raised-field agriculture is virtually unstudied. Literature on other ecosystems with frequently waterlogged soils – paddy rice systems in Asia (IRRI, 1985; Timsina and Connor, 2001), peatlands in Europe (Olde Venterink et al., 2002), flooded pampas in southern South America (Rubio et al., 1997) – suggests the interest of closing this gap in our knowledge.

Phosphorus and nitrogen, the nutrients most likely to be limiting to plant growth, also have the most distinctive behaviour under anaerobic conditions (Vasey et al., 1984). Waterlogging usually enhances the availability of phosphorus, making it more soluble and more diffusible, and flushing it from the organic P pool when soil microorganisms are killed under anaerobic conditions (Rubio et al., 1997). Low P availability is a frequent problem in highly weathered Ultisols and Oxisols, where P is adsorbed to iron and aluminum oxides. Low P availability is also a problem in a family of young volcanic soils, andisols, where P is adsorbed to allophane clays. We could find no information on how anaerobic conditions affect this sorption. In all these soils, waterlogging could also increase P availability simply by maintaining a large proportion of P in undecomposed organic matter, nutrients in which become available to plants when muck is deposited on raised fields, where conditions are aerobic. Waterlogged soils also offer much less resistance to root penetration, allowing plants to produce a greater proportion of fine roots that more efficiently acquire nutrients of low mobility, such as P (Rubio et al., 1997). Plants that are tolerant of waterlogged conditions may thus be able to extract more P from soils than can plants growing in better-drained environments. Rice is a good example. Its waterlogging-tolerant roots extract P efficiently, reducing the need to apply P to wetland rice (Timsina and Connor, 2001). While the crops pre-Columbian raised-field farmers grew were insufficiently adapted to waterlogging to have benefited directly from such effects, wild plants growing in the flooded matrix could have benefited, increasing the overall quantity of P cycling in the system. Detritus from these plants could have provided relatively P-rich mulch for addition to raised fields. Increased P availability through such mechanisms could be important, because P is often limiting to plant growth, and soils of at least some abandoned raised-field complexes have generally low contents of P (e.g., French Guiana, J.J. Birk and B. Glaser, unpublished results).

The dynamics of nitrogen add another level of complexity. Nitrogen availability is usually negatively affected by waterlogging (Timsina and Connor, 2001). Nitrogen accumulates in organic matter deposited in anaerobic conditions, and when this organic matter is transported into aerobic conditions, nitrogen is rapidly mineralized to nitrate. These dynamics are important in the supply of nitrogen to crops on raised fields (Vasey et al., 1984). As mentioned above, biological fixation of atmospheric nitrogen is usually favored only in systems where phosphorus is not limiting. Increased P availability in raised-field systems should thus also increase the rate of nitrogen fixation.

Nutrient dynamics in raised-field agriculture not only affect crop production, they also affect the ecosystem services that can be supplied by these systems. In experimental raised fields near Lake Titicaca, Carney et al. (1993) showed that the efficient use of nutrients by these systems had important environmental consequences. By retaining nutrients and sediments, rehabilitated raised fields reduced water pollution downstream. Raised-field agriculture thus conferred environmental benefits in addition to the economic benefits from crop production and maintenance of soil fertility. This example suggests that further studies of these farming systems as ecosystems could be rich with insights on how to reconcile agricultural productivity with ecosystem services.

Among the biggest gaps in our understanding of how raised fields may have functioned as agroecosystems is the virtually complete absence of work on microbial communities in these systems. Once again, work on paddy-rice systems (Roger, 1996) suggests exciting possibilities. Microbial biomass is an important potential source of nutrients for plants, and flooding leads to an increase in total microbial biomass, in large part attributable to aquatic microalgae. However, nutrients in living microbes are immobilized and unavailable to plants, becoming available only when the microbes die and the nutrients they contain are mineralized. Nutrient release thus depends on microbial turnover. This idea forms the base of one explanation of the “Birch effect”, i.e., the frequent observation that nutrient release increases in soils subjected to drying and re-wetting cycles (Jarvis et al., 2007). According to this idea, microbes that die as the soil dries are rapidly decomposed (and their nutrients mineralized) as decomposer populations rapidly increase with re-wetting.

The “Birch effect” and similar phenomena could be particularly significant in raised-field systems, where a diversity of mechanisms could enhance microbial turnover, mineralization and nutrient availability to plants. In these systems, seasonal nutrient flushes could occur not only when dry soils are re-wetted, but also when waterlogged soils become merely moist. For example, microalgae that accumulate during flooding die when floodwaters recede, and under moist, well-aerated conditions their nutrients may then be mineralized. Furthermore, in raised-field systems drying and rewetting cycles are produced not only by natural seasonal change, but also by human-mediated transfer of organic matter from flooded to well-drained compartments of the system. Biomass of aquatic microbes could lead to nutrient flushes as they are decomposed in raised-field soils. Similarly, nutrient release to plants could be enhanced by the accumulation of plant-derived organic matter under anaerobic, waterlogged conditions, followed by its rapid decomposition and mineralization once it is added to the well-drained raised fields.

These remarks are highly speculative, but what we know about paddy-rice systems (Roger, 1996) suggests that such speculation is not unfounded, and that studies of microbial ecology in raised-field agriculture are vital to understanding how these wetland agroecosystems functioned.

5.3.2.4 Functioning of raised-field systems at the landscape level: the role of fallow periods

Another large gap in our knowledge of how pre-Hispanic raised-field agriculture functioned is whether cultivation was continuous or whether fallows were incorporated, and if so, how field/fallow cycles were organized in space and time. These are critical points in assessing the productivity of raised-field agriculture both in terms of land demand and of required labor input, and the level of use that is sustainable. The archaeological record is virtually silent on these points. Carbonized wild plant seeds present in ancient raised fields of Tiwanaku, interpreted by Erickson (1994) as possibly the result of burning of llama dung, could also reflect the burning of fallow vegetation during the preparation of a new cultivation cycle (Gondard, 2006). Experiments with rehabilitated raised fields give some insight. Based on results of these experiments, Erickson (2003) considers that under good management, raised fields at Tiwanaku may not have required long fallow cycles. In some of these rehabilitation experiments, however, yields have been reported to decrease after 3–4 years, leading to the claim that fallows would have been required (Bandy, 2005). Bandy (2005) has suggested that fallows could have been necessary at Tiwanaku to limit damage done by potato root nematodes. However, several objections can be raised to this hypothesis. First – and this is a general problem with the experiments that have been done – virtually the only thing that is measured in these experiments is yield. We have no clue as to what variables (e.g., pressure from pathogens or weeds, declining nutrient availability) might explain variation in yield. Second, there is no evidence for the importance of these pathogens in these sites. Third, pre-Hispanic farmers may have managed field environments, crop populations, or both, in ways that obviated any need for fallow. Contemporary farming systems that are more or less analogous certainly include examples of successful permanent cultivation over very long periods. The best-known, and probably most durable, example is the *chinampas*. They have been cultivated virtually continuously for centuries. While they are essentially a lacustrine system and thus differ in many ways from extinct systems, most of which were in seasonally flooded environments, they give interesting insights into the questions we examine here. *Chinampa* soils contain several fungal species that limit proliferation of crop pathogens, including both nematodes (Zuckerman et al., 1994) and fungi (Lumsden et al., 1987). High organic matter content and greater biological activity are thought to favor the pathogen-suppressive activity of *chinampa* soil. However, fallows frequently do reduce the incidence of soil-borne crop pathogens. Mulching of raised fields with green manure from slashed vegetation from the fallow could contribute to this effect, because plants also contain diverse pathogen-suppressive metabolites (Chitwood, 2002).

Rehabilitation experiments have simply not been carried out over long enough periods to determine whether permanent cultivation was possible, and they have not measured agroecological parameters that could help us interpret their results. It seems likely that not all raised-field systems could have been cultivated continuously. First, some were characterized by soil of markedly lower fertility than in the Basin of Mexico, where *chinampas* were continuously cultivated. Second, most were located in highly seasonal environments, and were "fallow" each year for at least much of the dry season (though how long is unclear). Third, raised-field complexes were likely not all built at the same time; a cycle of building, use and reconstruction may have existed (Darch, 1988). Fallows may have been an integral part of such cycles. Fourth, fallows are included in some extant raised-field systems in Asia and Oceania. On Uvea, western Polynesia, "garden-island" raised fields are left fallow for unspecified periods after several successive cropping periods (Kirch, 1978). After fallow, they are rebuilt and the cut herbaceous fallow vegetation is incorporated into the soil. Significantly, only a single area of the island with exceptionally good soil was cultivated continuously.

As in other agroecosystems, multi-year fallow periods between cultivation cycles in raised-field farming systems could have played roles in restoring soil fertility and in escaping pests. In tropical forest environments, restoration of soil fertility during fallow periods is usually associated with substantial buildup of plant biomass, the source (after slashing and burning) of most of the nutrients that permit growth of crops. In seasonally flooded savannas, biomass accumulates at much lower rates. However, traits peculiar to wetland environments may have influenced the potential of fallow periods to restore soil fertility. During fallow periods, for example, wild plants adapted to waterlogging probably grew more abundantly than when fields were actually in use. These plants are probably better than the upland crops grown in raised fields in extracting P from waterlogged soil and capturing it in biomass or storing it in soil organic matter, which could be added to raised fields in a new cultivation cycle.

Among the variables affecting the quantity of nutrients that could be stored during fallow periods, frequency and intensity of fires may be among the most important. Fires mineralize N, P and other nutrients. Although some loss occurs through erosion, savannah soils appear quite efficient at retaining nutrients released by fire (Kellman et al., 1985). However, fires also result in substantial loss to the atmosphere of N (through volatilization [Wan et al., 2001]) and of P (as aerosols [Mahowald et al., 2005]). Frequent fires could also reduce vegetation cover and root density, lowering the efficiency of the plant community in capturing nutrients, and in storing them underground where they are less susceptible to loss through fire (Kaufmann et al., 1994). Frequent, intense fires could thus severely impact long-term primary productivity and limit the accumulation of soil organic matter. Savannas are fire-prone environments, and limiting the frequency and intensity of fires may have been an important aspect of the management of fallows by raised-field farmers.

5.3.2.5 Self-organizing processes in fallow raised-field complexes

As McKey et al. (2010) outlined, self-organizing processes could have greatly enhanced the capacity of fallow periods to restore soil fertility in maintaining human-initiated concentration of resources on the raised fields during fallows. Our data from "fossil" raised-field landscapes of French Guianan coastal savannas show that many organisms that have important "engineering" activities, notably social insects, earthworms and woody plants, are strongly concentrated on the well-drained soils of abandoned raised fields. Their actions – transport of organic and mineral matter to mounds, modification of the properties of soils of abandoned raised fields in ways that reduce their erodibility – can contribute to compensate the non-negligible erosion we have demonstrated in this landscape (McKey et al., 2010). Thus the concentration of resources on raised fields initiated by humans continues after field abandonment (e.g., in fallows), driven by engineering activities of other organisms. Such engineering activities appear to have contributed to maintaining pre-Columbian raised fields for centuries following their abandonment. Such "outsourcing" of the task of resource-concentration to natural engineers could also have reduced the labor costs of restoring raised fields in each cultivation cycle, contributing to the resilience of the system. The limited information available suggests similar dynamics in other raised-field systems. In the Llanos de Mojos, termite nests are concentrated on abandoned raised fields (see Plate 14b in Denevan, 1966).

The dynamics described by McKey et al. (2010) concern raised-field landscapes that were abandoned centuries ago. It is not known how long it took for such dynamics to be established after raised fields were abandoned. How long did it take for organisms to colonize abandoned raised fields, and in what sequence did they colonize? How much did abandoned raised fields erode before engineer-generated feedbacks kicked in? Alternatively, were at least some organisms with marked engineering activities already present in active fields?

According to Byers et al. (2006), "manipulating biotic interactions to provide desired services and thus reduce or eliminate the need for external inputs is fundamental to the practice of ecologically sound agriculture." Positive feedbacks between the actions of the farmers who constructed mounds and those of the organisms that maintain them illustrate an often strived for, but frequently elusive, objective of ecological engineering: exploiting synergies between actions of human engineers and those of natural ecosystem engineers, so that ecosystems self-organize to fit with technology (Odum and Odum, 2003).

5.3.2.6 Functioning of raised-field agriculture: socioeconomic organization

Knowing why past cultures adopted raised-field agriculture, how these farming systems were organized socially, technically and economically, and why they were eventually abandoned, could help us assess whether it is feasible and desirable to re-introduce some of their elements today. Here again there is great debate and substantial uncertainty. The debate focuses mostly on the system most intensively studied by archaeologists, the enormous area of abandoned raised fields around Lake Titicaca, in Peru and Bolivia, which were cultivated beginning about 3000 years ago and formed much of the subsistence basis for the Tiwanaku empire (Kolata, 1996).Why did raised-field agriculture in this area originate? Some archaeologists view raised-field agriculture as a textbook example of the process of induced intensification treated in the influential work of Ester Boserup (1965). According to her theory, people initially practiced a more extensive type of agriculture requiring relatively little labor. With increasing population pressure, these extensive methods no longer met food demands. People thus had no other choice than to adopt more time-demanding intensive farming systems (Bandy, 2005).

According to another view, forced labor imposed by a governing elite to produce surpluses may have been as important as “population pressure” in driving agricultural intensification. Top-down hierarchical control by a central authority may have been necessary to organize the construction and maintenance of raised fields and associated earthworks (Kolata and Ortloff, 1989). This view also suggests hypotheses about why raised-field systems were abandoned, considering their fall as the inevitable consequence of the collapse of the empires on which they depended. Collapse may have been triggered by environmental change, by forces internal to the society, or by the two in combination.

Erickson (1992) offers a contrasting viewpoint on all these points. According to his view, people adopted raised-field farming driven by preference, not by necessity. For example, in the altiplano, people may have preferred to live in lakeshore environments because they were richer in wild resources, and adopted raised-field agriculture as the best way to farm in the kinds of places they preferred to live. In keeping with this idea, no strong central authority would have been necessary to develop and maintain earthworks. As pointed out above (see Morris, 2004), these were not irrigation systems that required control of water allocation. Each set of raised fields functioned largely independently. Hypotheses about strong top-down control in “hydraulic societies” may thus have little pertinence here (Stanish, 1994). “Bottom-up” cooperation among family groups or neighbors would have been sufficient to construct and maintain earthworks. Again in keeping with these ideas, raised fields continued to be farmed, at a smaller scale, after the collapse of the Tiwanaku empire (Gray, 1992).

Answers to all these questions can help us assess the desirability and feasibility of rehabilitating raised-field agriculture in the 21st century. Systems requiring strong top-down control are likely to be both unfeasible and undesirable. Systems in which increased productivity per unit land comes at the cost of greatly increased labor per unit of yield, without a corresponding increase in the valuation of labor, is also not a desirable future. Raised-field farming can be part of a sustainable future only if people choose to adopt it because it creates viable rural livelihoods.

5.4 Raised-field agriculture: is it pertinent to today's world?

Is it desirable, and feasible, to attempt to rehabilitate ancient raised fields, or to promote the development of new raised-field agriculture in similar environments?

5.4.1 Why promote further agricultural conversion of wetlands?

Wetlands are important in biodiversity conservation and contribute significantly to global ecosystem services. Wetlands are everywhere under threat, and the principal threat is conversion to agricultural use (Daniels and Cumming, 2008). Why make this problem worse than it already is? Two points are relevant to this question. First, many wetlands are already degraded. Raised-field agriculture could be integrated into projects aimed at restoring their value for conservation and for providing ecosystem services. Second, maintaining "pristine" wetlands that exclude humans is unlikely to be an option, except for a relatively few integrally protected areas. Even globally important wetlands such as the Pantanal are under enormous pressure (Junk and Nunes de Cunha, 2005). In the view of one writer, "if wetlands are to be maintained in some semblance of ecological and hydrological health, they will have to be utilized for both traditional and modern sustainable uses (even to the extent of some degradation) or they will be lost" (Smardon, 2006). Aside from putting wetlands under rice monoculture, most attempts to develop agriculture in wetlands have envisaged draining them for cattle ranching or for planting upland crops. Agriculture has usually worked against wetlands rather than with them (Rijsberman and de Silva, 2006). By working with wetlands, raised-field agriculture could contribute to reconciling food production and environmental quality (Robertson and Swinton, 2005), creating "working wetlands" that meet multiple needs (Dialogue on Water, Food and Environment; www.iwmi.org/dialogue) and "floodplain-friendly" development alternatives (Junk and Nunes de Cunha, 2005). Raised-field agriculture, modern agroecology based on it, and culturaland ecological-based tourism activities aimed at experiencing and interpreting culture/environment interactions from the perspectives of past peoples could be integrated into sustainable use schemes (Smardon, 2006).

Climate change stands to have dramatic effects on tropical wetlands (Mitsch et al., 2009). However, in environments where raised-field agriculture remains possible, it could help reduce greenhouse gas emission and thereby combat climate change. By working with wetlands, instead of against them, raised-field agriculture could help maintain tropical wetlands and their soils, which lock away large quantities of carbon from the atmosphere for millennia (Jungkunst and Fiedler, 2007). Seasonal tropical wetlands are fire-prone environments (Mitsch et al., 2009). By limiting fire during fallows, raised-field farmers could further reduce carbon emissions. Furthermore, fallow raised fields provide habitat for flooding-intolerant components of wild biodiversity.

Raised-field agriculture could have further environmental benefits. In the Beni savannas of the Llanos de Mojos of Bolivia, work carried out by Oxfam and the Fundación Kenneth Lee has shown that in addition to providing a form of alternative food security to the poorest and most vulnerable segments of the region's rural populations, raised-field agriculture could reduce the environmental and economic impact caused by the severe flooding regime of the Mamoré and Beni rivers associated with strong La Niña events (CEPAL, 2008; Latrubesse et al., 2010). As in the Lake Titicaca basin (Erickson, 2003), raised fields help put crops beyond the reach of devastating floods. However, flooding regimes are more variable in these dynamic river basins than in lacustrine environments, and extreme floods would still impact lower-lying fields. Finally, raised-field agriculture offers an alternative to the clearing of tropical forest for slash-and-burn agriculture. Preliminary results of rehabilitation experiments indicate that raised-field systems reduce risks associated with floods and are extremely productive, producing two to three maize crops per year with annual yields of up to 5 t ha^{-1} (Saavedra, 2009). However, we are just beginning to understand how these agricultural systems function within the context of the larger fluvial environment and these experiments are still small-scale and short-term, preventing proper assessment of their sustainability.

5.4.2 What factors could limit the adoption of this way of practicing agriculture?

Some critics of attempts to rehabilitate raised-field agriculture consider them to have failed (Bandy, 2005; Chapin, 1988). Scholars who expressed early enthusiasm are now quick to point out that any lessons pre-Columbian raised-field farming has for agriculture today must be very carefully drawn (Denevan, 2001; Siemens, 2004). However, the difficulties encountered in attempts to rehabilitate raised-field farming are simply examples of a problem faced in any move to ecological agriculture today: "Today's world is filled with urban workers while what we need are rural ecologists... the destruction of rural society has taken with it the knowledge base and labor force that will be needed for the transformation" (Vandermeer, 2011). If ecological agriculture is to be successful anywhere, these paradoxes have to be resolved.

The most comprehensive analyses of why most farmers involved in rehabilitation attempts have not permanently adopted raised-field farming as a production strategy are offered by the hardiest champions of these experiments. Both Erickson (2003) and Gliessman (1991) cogently discuss the sustainability of raised-field agriculture, pointing out that the problem is not simply one of technology, soil fertility, or labor requirements, but includes complexities that other critics have not acknowledged. High labor cost of construction is often cited as a principal drawback of raised fields (e.g., Bandy, 2005). However, although the initial construction of large blocks of raised fields requires considerable labor, total labor may be relatively low when spread out over years of cultivation (Erickson, 2003; Mathewson, 1987; see Section 5.4.3), particularly if ecosystem engineers contribute to raised-field maintenance during fallows (McKey et al., 2010). "The most important factor explaining non-adoption by farmers", Erickson (2003) concludes, "is that the social, political, and economic environment today is different from that when the raised fields were first constructed and used." For example, competing labor demands exist today that reduce the opportunity for initiating raised-field construction. Furthermore, livestock is now a significant source of income for Quechua farmers, who must decide between using land for rehabilitating raised fields or for grazing livestock. Political instability also led to irregular funding for rehabilitation experiments, leading to abandonment of many fields.

Chapin (1988) and Gliessman (1991) present strongly contrasting analyses of rehabilitation experiments in Mexico. According to the former author, the seductive *chinampas* model from the highlands was inappropriately transposed into lowland environments in Veracruz and Tabasco: "In both cases, the stated and unstated objectives of project managers had little fit with the interests and needs of the farmers. The two projects were designed and implemented by outside technicians without significant local participation, and both rapidly fell apart when 'beneficiaries' failed to cooperate" (Chapin, 1988). Gliessman (1991), who did extensive field work in the area on a third project not mentioned by Chapin (1988), paints a very different picture. Projects in which agroecologists cooperated with local people had begun to accumulate the data needed to adapt the highland model to lowland environments (e.g., Boucher et al., 1983; Gliessman, 1992). The experiments showed that achieving high yields would take time: newly built platforms took 2 years to accumulate adequate levels of organic matter and available nutrients and a further 3 years or more to develop good agricultural soil. Unfortunately, these experiments were terminated too soon. Inspired by these fledgling projects, government officials decided to initiate a large-scale program. According to Gliessman (1991), this last project (which was the principal target of Chapin's [1988] critique) was top-down in its approach and generally poorly designed. Before failing, it led to reduced funding and interruption of the promising smaller projects, bringing them down as well.

5.4.3 Labor requirements and the potential of raised-field agriculture to support viable rural livelihoods

The pertinent question about raised-field agriculture is not whether it can work in today's world, but whether it can work in tomorrow's world. How agriculture is conducted must be radically rethought. There are currently few signs that governments are ready to encourage food production strategies that help mitigate and adapt to climate change, conserve biodiversity and achieve social justice. It is thus more important than ever that ecologists continue to argue for the necessity of these goals, and test ideas about how to accomplish them. Does raised-field farming have a place in the new kinds of agriculture that must emerge?

The labor-intensive nature of raised-field farming has been considered a major drawback to its adoption. However, the amount of labor that was actually needed to construct raised-field systems is unknown. In archaeology, estimating time and labor costs of constructing raised fields and other earthworks is a difficult enterprise, fraught with uncertainty concerning many variables, including the volume of earth moved, the amount of work required to do it with the tools available, the number of people available to do the work, how long their workdays were, and how much maintenance the system required once constructed. Researchers vary in the attention they give to each of these variables (estimates for each are not always made explicit), and estimates are expressed in different ways, rendering comparison difficult. Arco and Abrams (2006) estimate that construction of the entire *chinampa* system in the Basin of Mexico (6500 ha) took 25 million worker-days, spread over 40 years. This amounts to 1712 worker-days per year, but the size of the labor force was not estimated. Mathewson (1987) estimated that the labor necessary to produce and maintain the 50,000 ha of raised-field complexes in the Guayas Basin, Ecuador, amounted to only 12 days per year per family from the start of the system (1600 B.C.) to its end (1500 A.D.). Turner and Harrison (1983) estimate that between 710 and 3266 worker-years were required for construction of the 310 ha of raised fields in Pulltrouser Swamp. Assuming a labor force of 100 workers, construction would have taken from 7.1 to 32.7 years. Using a wide range of estimates, Walker (2004) argues that the raised fields in the Llanos de Mojos could have been constructed by a small number of people. For example, he estimates that the largest of the raised fields in northwestern Mojos, which cover about 1.8 ha each, could each have been built by a group of 100 people in a single episode of 20 days.

It is interesting to compare these estimates with construction costs that have been measured for a present-day Asian system that is a fairly close analogue. The sorjan cropping system of Indonesia combines growing upland crops on raised beds and lowland crops in the sink (IIRR, 1990). In a trial experiment, constructing the raised beds and sinks (50 cm from the bottom of the sink to the top of the bed; beds 3.5 m wide, sinks 3 m wide) required 4479 worker-hours of labor per ha of sorjan (Domingo and Hagerman, 1982). Assuming a 6-h workday, this amounts to 746 worker-days per ha. Again, while comparison is difficult, these measured values appear substantially lower than most of the archaeologist's estimates. Although this initial labor cost may still be considered high, once constructed sorjan beds are fairly permanent structures. Their construction should thus be considered as an investment rather than a simple labor cost. Labor requirement could be reduced by the use of earthmoving machines to construct raised fields, as in some rehabilitation experiments (Denevan, 2001; Saavedra, 2009), but care must be taken to avoid destroying by compaction the physical properties that help make soils favorable for agriculture (Chapin, 1988).

If we succeed in devising mechanisms by which farmers are justly compensated for labor that provides ecosystem services in addition to food production, then labor-intensiveness may no longer be an obstacle. It could in fact confer important advantages, providing jobs for rural populations and thereby reversing the exodus to cities driven by the lack of gainful rural employment in industrial agriculture.

The *chinampas* of Mexico City provide an instructive example. After many centuries, the system is still part of the region's agricultural picture, although it is much altered, even degraded, and under threat (Torres-Lima et al., 1994). Adapting to modern pressures, *chinamperos* grow as their most important crop cut flowers, rather than staple foods. In addition to research on the ecological principles applied in management – a classical theme in *chinampas* research (Armillas, 1971; Coe, 1964) – what is needed now is understanding of the relationships between agroecological principles and socioeconomic strategies addressing urban development, regional employment, and environmental concerns (Torres-Lima et al., 1994).

5.5 Conclusion

Raised-field agriculture in the Neotropical region could yet be resurrected. Data from archaeological and contemporary raised-field systems suggest how their functioning at the local plot scale fits food production to ecosystem processes, but experiments conducted so far to determine the feasibility of re-establishing such systems have encountered a number of methodological problems. Their focus has been on measuring yield, rather than studying the ecological interactions that underpin yield. Furthermore, the agroecological diversity of raised-field systems has not been appreciated. Raised-field systems function differently under different soils and climates. Except for those in the richest environments, fallow periods are probably necessary, but experiments have not been conducted over long enough times to assess the importance of fallows and the roles they play. Experiments have also been too short to explore how raised-field agriculture can be adapted not only to local biophysical environments but also to social, economic, and political contexts – or how the social capital necessary for ecological agriculture can be reconstructed.

We believe that two distinct kinds of experimental studies are needed. First, there is still a great need for research aiming simply to fill the large gaps in our knowledge about how pre-Columbian raised-field farming systems may have worked, without pressure to demonstrate their usefulness in some real-life situation today within the time frame of a typical 3- to 5-year project cycle. There is great diversity among systems, and great depth within each. We have barely scratched the surface, and this knowledge will be necessary for experiments of the second type, those aimed at re-establishing raised-field agriculture as a sustainable food-production system today and in environments in the near future in which resources such as land, fertilizers and water will be more limited. In research aimed at clarifying how raised fields worked (and work), we must look further afield than we have so far for inspiration from present-day systems. Dynamic sorjan cropping systems in Asia may have as much to tell us today as the *chinampas*. Detailed studies of energy and nutrient flow in highly integrated present-day field/pond agroecosystems in southern China (Guo and Bradshaw, 1993; Luo and Lin, 1991) may yield more insights than the coarse-grained inferences possible from archaeological and archaeobotanical studies. We must understand the agroecology of raised-field farming systems in enough detail that, instead of proposing a preconceived, prepackaged model to farmers, we will be able to propose *à la carte* those pieces that might be suited to their needs in any one of a diversity of situations today.

For experiments aimed at re-establishing raised-field agriculture as a sustainable food-production system, the single overriding lesson from previous work is that the people affected by these experiments must be involved in the fundamental decisions from the start. The solutions that emerge must fit their needs, not the other way around. The labor-intensive nature of raised-field agriculture may make it unsuitable in many present-day contexts in Latin America, but if conditions are created that favor ecologically sustainable intensification of agriculture by reconstructing the social capital on which such agriculture rests and by compensating the labor of farmers who conduct it, raised-field agriculture could have broad application. Rigorous studies of raised-field agroecology are required if we are to be ready.

Chapter 6

Legacy of pre-Columbian raised-field agriculture in French Guiana on soil organic matter and nutrient concentrations

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6.1 Abstract

Remains of pre-Columbian raised fields cover vast areas in the periphery of Amazonia. Wetland agriculture on artificial raised mounds and ridges allowed cultivation in areas subjected to regular flooding. Today, the remains of pre-Columbian raised fields in coastal savannas of French Guiana provide habitats for plants, termites, ants and earthworms, which appear to stabilize the raised structures against erosion. In maintaining suitable habitats for themselves and other organisms, they also preserve a physical legacy of pre-Columbian agriculture. It is suspected that raised-field agriculture supported high human population densities in pre-Columbian periods. However, raised fields in French Guiana are found in areas that are generally nutrient-poor. How farmers managed nutrients and organic matter in raised fields, and the effects of their management on nutrient availability in the past and today, are unknown. Knowledge on these points is crucial for estimating population densities that could have been supported. We studied contents of nutrients and total organic carbon in abandoned raised-field sites in French Guiana to assess the biogeochemical legacy of soil management by pre-Columbian farmers. At five raised-field sites, amounts of total organic carbon, total nitrogen and plant-available nutrients were analysed. Additionally, at two sites, pseudo-total element concentrations (extracted with aqua-regia) were analysed. Amounts of total organic carbon were elevated in some raised fields in comparison to adjacent flat areas. The concentration of pseudo-total phosphorus was correlated with the amount of total organic carbon. Slightly elevated concentration of plant-available phosphorus was found in some raised fields in comparison to adjacent flat areas, but the concentration of plant-available phosphorus remained overall low. The slight enrichments observed could have been caused by construction of raised fields using topsoil, by their maintenance or by plant material applied to soils by pre-Columbian farmers, and by the effects of soil invertebrates. Our examination of the biogeochemical legacy of pre-Columbian raised-field agriculture did not provide clear evidence that farmers fertilized the raised fields with particularly nutrient-rich materials. Farming these nutrient-poor soils (other than cations originating from brackish groundwater) probably required long fallow periods, supporting population densities that were lower than in most current estimates.

6.2 Introduction

During the last decades, evidence has increased showing impacts of pre-Columbian societies on Amazonian ecosystems (Denevan, 2001; Erickson, 2008; Glaser et al., 2001b; Heckenberger and Neves, 2009; Iriarte, 2007; Petersen et al., 2001; Rostain, 2012). The extent to which Amazonian ecosystems, prior considered as 'pristine', are the legacy of pre-Columbian land use is currently highly debated (Barlow et al., 2012; Clement and Junqueira, 2010; Levis et al., 2012; McMichael et al., 2012). The idea of widespread human occupation of pre-Columbian Amazonia has inspired optimism that research on modification of ecosystems by pre-Columbian people could provide knowledge to develop sustainable agriculture in the humid tropics (Glaser, 2007; Heckenberger et al., 2008; Renard et al., 2012b; Sombroek et al., 2003; Woods and McCann, 1999).

Vestiges of pre-Columbian raised-field agriculture cover vast areas of seasonally flooded savannas in the periphery of Amazonia. Plant cultivation on artificially raised surfaces allowed agriculture in these areas. Raised fields have been found in the Llanos de Mojos of Bolivia (Denevan, 1966; Erickson, 1995; Saavedra, 2009; Walker, 2004), in the Llanos of Venezuela (Denevan and Zucchi, 1978; Spencer, 2000; Zucchi and Denevan, 1972) and in the coastal savannas of the Guianas (Rostain, 1991, 2008a, 2010; Versteeg, 2008). In French Guiana, raised fields range from large ridges usually 0.3 – 0.8 m high, 1 – 4 m wide and 5 – 30 m long, to small, round mounds usually 0.5 – 1.5 m diameter and 20 – 50 cm high (McKey et al., 2010; Rostain, 2008a, 2010). Raised fields in French Guiana are associated with the Barbakoeba and Thémire cultures, which flourished between ca. A.D. 950 and A.D. 1150 and A.D. 1150 and A.D. 1450, respectively (McKey et al., 2010; Rostain, 2008b).

Centuries after being abandoned, raised fields in French Guiana still persist, despite being subject to highly erosive tropical rainfall and to fires that seasonally reduce vegetation. McKey et al. (2010) and Renard et al. (2013) provided evidence that re-engineering by non-human organisms has enabled them to persist. According to this idea, raised-field farmers acted as ecosystem engineers by creating raised fields. These patches of soil remain above the water level during seasonal flooding, providing habitats for soil ecosystem engineers, mainly termites, ants, earthworms and plants. By transporting material to the raised fields and by stabilizing them against erosion, these ecosystem engineers maintain the raised structures. Thus, vestiges of raised fields in French Guiana may be examples of coupled systems of human and natural engineering. The physically structured raised-field areas are described by McKey et al. (2010) and Renard et al. (2013) as alternative stable states of marshy savannas. The savannas would have remained in a stable state (relatively flat and homogeneous) had raised fields not been constructed. After being abandoned, the raised fields would have eroded and the savannas would have returned to the flat state if the raised structures were not maintained by the ecosystem engineers. McKey et al. (2010) suggested that pre-Columbian raised field agriculture in coastal savannas of French Guiana could serve as a model for how self-organizing processes in coupled human and natural systems could be exploited in agriculture.

Although concepts regarding the physical modification of the savannas by raised-field farmers and its long-term effects have been elaborated, little is known about the biogeochemistry of raised fields. The management of organic matter and nutrients by pre-Columbian raised-field farmers as well as its long-term biogeochemical effects remain unknown (Renard et al., 2012b). Raised fields in French Guiana are found in areas where soils have low nutrient concentrations (Boulet, 1975; Leprun et al., 2001). It is therefore difficult to imagine the existence of dense pre-Columbian populations, dependent on raised-field agriculture in this area, as proposed by Rostain (2008a, 2010), Iriarte et al. (2010) and McKey et al. (2010), without some way of efficient nutrient management. Knowledge of pre-Columbian nutrient management is also crucial for understanding the feasibility of re-establishing this technique today.

The plant availability of nutrients in raised fields could be affected by agricultural techniques (Renard et al., 2012b), by natural ecosystem engineers (Chapuis-Lardy et al., 2011; Decaëns et al., 2001; Frouz and Jilková, 2008) and by their combined activities. Nutrient enrichment in raised fields could be possible at a small scale through translocation of nutrients from the flooded basin to the raised fields by raised-field farmers (Renard et al., 2012b) and by natural ecosystem engineers (McKey et al., 2010). However, a substantial enrichment leading to very high amounts of nutrients in an area of otherwise nutrient-limited soils is hard to envision via these small-scale translocation processes. Higher nutrient enrichment would be possible by fertilization with material originating from external sources.

In Meso- and South America, fertilization seemingly was a component of pre-Columbian agriculture, including raised-field agriculture. Reviewing the evidence, Denevan (2001) considered that fertilization with faeces was probably of major importance in Andean agriculture, and some soil micro-morphological features in raised fields in Ecuador indicate the application of animal manure (Wilson et al., 2002). In

Mesoamerica, compost, probably including faeces, was used to fertilize raised fields in the Valley of Mexico (Armillas, 1971). On the Pacific coast of South America, several reports exist of fish being used as fertilizer in addition to guano (Denevan, 2001). Reports from the sixteenth-century describe that the Incas transported topsoil to their fields from a distance (Denevan, 2001). For a complex of raised fields in French Guiana (K-VIII site), McKey et al. (2010) and Iriarte et al. (2010) found signs that raised fields were built with material mainly transported from a higher, less flooded area < 100 m from the raised fields. However, little is known about how widespread and important soil fertilization was in pre-Columbian agriculture in Amazonia. Deposition of nutrient-rich material, e.g. bones (Lima et al., 2002; Schaefer et al., 2004) and faeces (Birk et al., 2011), contributed to the genesis of Amazonian Dark Earths in central Amazonia, but no clear evidence was found showing that pre-Columbian farmers in central Amazonia intentionally applied these materials on soils to fertilize agricultural fields at large scales (Glaser, 2007; Glaser and Birk, 2012; Lehmann, 2009; Sombroek et al., 2003; Woods and McCann, 1999).

A fertilization-induced increase of the stocks of soil organic matter (SOM), and of phosphorus and other nutrients, can persist in the soil for long periods of time (McLauchlan, 2006; Oonk et al., 2009). The example of Amazonian Dark Earths shows that pre-Columbian soil modifications by the deposition of organic materials and particularly nutrient-rich materials can lead to increases in the amounts of SOM and total and plant-available nutrient concentrations in Amazonian soils that still persist, at least in well-drained areas (Glaser and Birk, 2012; Glaser et al., 2001b; Kämpf and Kern, 2005; Smith, 1980; Sombroek, 1966; Zech et al., 1990).

The aim of this study was to analyse if the physical legacy of persistent raised fields in French Guiana is accompanied by a soil chemical legacy of higher SOM and nutrient concentrations. The study focused on nutrient and total organic carbon (TOC) concentrations in raised fields to analyse if effects of pre-Columbian fertilization or other concentration processes are detectable in raised fields today. Therefore, raised fields belonging to all types of mounds and ridges previously described in French Guiana (McKey et al., 2010; Rostain, 2008a, 2010) were sampled at five sites and their properties compared to those of soils in flat savanna areas in the surrounding area. TOC and total nitrogen concentrations were analysed. Plant-available phosphorus, a classical indicator of human deposition of nutrient-rich materials (Bethel and Máté, 1989; Holliday and Gartner, 2007), was also measured. A multi-element extraction technique was used that allows simultaneous analysis of plant-available calcium, potassium, magnesium and sodium. To evaluate the influence of acidity on nutrient availability, pH was measured.

At two sites, pseudo-total concentrations of phosphorus, calcium, potassium and magnesium were additionally measured to extract these nutrients from more stabilized pools. Pseudo-total element extraction do not completely extract all elements, e.g. tectosilicates and are not completely dissolved (Blume et al., 2000; Schwartz and Kölbel, 1992). However, they result in complete extraction of anthropogenically deposited elements in most cases (Blume et al., 2000; Linderholm and Lundberg, 1994; Wilson et al., 2006). Copper, zinc and barium were measured in these extracts to get more detailed information about the materials that could have been applied by pre-Columbian farmers.

6.3 Material and Methods

6.3.1 Study area

The raised fields in French Guiana are located in savannas close to the coast. The climate in this region is seasonal humid tropical; mean annual temperature is 27 °C and mean annual rainfall is 3000 mm (Barret, 2001). Two rainy seasons occur: a long, intense rainy season from April to July and a less intense rainy season from mid-November to mid-February (Barret, 2001).

The recently formed coast of French Guiana is characterised by mud banks, mangroves and salt marshes (Turenne, 2010). The raised fields occur close behind the coast, in shallow basins bounded by elevated sandy *cheniers* (ancient beach ridges) that run roughly parallel to the present shoreline (Brinkman and Pons, 1968; Prost, 1989). These basins are regularly flooded with fresh water during the rainy seasons. During the dry seasons, they dry to a varying extent and fires are frequent (Renard et al., 2012a). Vegetation in relatively well-drained areas of the savannas where raised fields were found are dominated by Poaceae (*Paspalum pulchellum* Kunth) and Cyperaceae (*Rynchospora filiformis* M. Vahl and *Rynchospora globosa* Kunth) (Renard et al., 2012a). Relatively wet areas are mainly covered by Cyperaceae (*Eleocharis* sp. and *Cyperus* sp.).

The basins where raised fields are located are covered by Pleistocene (Coswine series, higher areas) and Holocene (Demerara series, lower-lying areas) fluviomarine and marine sediments (Boulet, 1975; Brinkman and Pons, 1968; Prost, 1989; Turenne, 1979). Their texture is typically clay and silty clay (Prost, 1989), but layers with higher sand content were observed during field work. Kaolinite and illite are the most abundant clay minerals in the Holocene sediments, with the former being predominant (Blancaneaux, 1981; Brugiere, 1975; Marius and Turenne, 1968). Montmorillonite is present in lower amount and small amounts of vermiculites and chlorites are also present in the Holocene sediments (Blancaneaux, 1981; Brugiere, 1975; Marius and Turenne, 1968). In other soils developed on Pleistocene sediments, kaolinite was found to be the most abundant clay mineral, and the content of other clay minerals is much lower than in the Holocene sediments (Karl, 2008).

All soils in the seasonally flooded savannas are influenced by water saturation to varying extent and show redoximorphic features (Boulet, 1975; Leprun et al., 2001; Misset, 1967; Quesada et al., 2011; Turenne, 1973), e.g. gleyic colour patterns and plinthic and ferric horizons (terminology in this article follows FAO [2006] and the IUSS Working Group WRB [2006]). High pyrite concentrations and thionic horizons can be found in relatively wet areas (Leprun et al., 2001). In some areas of the savannas, the brackish groundwater enhances salt contents in soils (Boulet, 1975; Misset, 1967). Clay migration is widespread and especially characteristic for soils developed on Pleistocene sediments (Misset, 1967; Turenne, 1973), causing the formation of argic and natric horizons. In very wet areas, accumulation of organic material at the surface forms histic horizons, locally known as ‘pégasse’ (Boulet, 1975; Leprun et al., 2001).

The pH value is typically between 4 and 5 (Boulet, 1975; Misset, 1967). An elevated pH value can be found in areas with high salt concentrations, and pyrite oxidation can cause pH < 4 (Boulet, 1975; Leprun et al., 2001; Turenne, 1973). The potential cation exchange capacity in soils developed on Pleistocene sediments is typically $< 20\ \text{cmol}_{c+}\ \text{kg}^{-1}$ and in the upper part of soils with clay migration $< 10\ \text{cmol}_{c+}\ \text{kg}^{-1}$ (Boulet, 1975; Misset, 1967). Soils with high SOM content and soil developed on Holocene sediments can show a higher cation exchange capacity of 25–30 $\text{cmol}_{c+}\ \text{kg}^{-1}$ (Boulet, 1975; Leprun et al., 2001; Misset, 1967; Turenne, 1973). The base saturation strongly depends on salt concentration; values < 15% are found where salt concentrations are low (Boulet, 1975; Misset, 1967; Turenne, 1973). The amounts of soluble and/or exchangeable Na, Mg, Ca and K are elevated in soils with enhanced salt contents (Misset, 1967; Turenne, 1973). Mg dominates at the exchange complex over Ca, K and Na in most soils and Mg and/or Na dominate at the exchange complex over Ca and K in soils with high salt concentrations (Boulet, 1975; Leprun et al., 2001; Misset, 1967; Turenne, 1973).

6.3.2 Sampled raised fields and flat areas

6.3.2.1 Selection of raised fields and flat areas

At five sites, all known types of raised fields in French Guiana were sampled. When possible, sites which were previously described (Iriarte et al., 2010; McKey et al., 2010; Renard et al., 2012a; Rostain, 2008a, 2010) were sampled in order to avoid confusion with raised structures that are not remains of pre-Columbian agriculture (McKey et al., 2010). Information on the genesis of the raised fields, on the archaeological background and on the distribution of raised fields is summarized in the supporting information (see SI1 in Appendix 2.1). Flat areas were sampled at four sites for comparison. However, large parts of the savannas are covered by raised fields. Flat areas that fulfilled at least two criteria—namely (1) an elevation that was similar to that of the raised fields and (2) a parent material that was similar to that of the raised fields—were hard to find. If no appropriate flat area with a texture similar to that of raised fields could be found, flat areas with higher clay content were preferred over flat areas with higher sand contents, in order to avoid overestimation of TOC and nutrient enrichment in raised fields. Nevertheless, texture could have been influenced by raised-field farmers by various processes, e.g. by transport of material from areas with differing texture to build the raised fields (Iriarte et al., 2010; McKey et al., 2010). Also, soil texture can be directly influenced by soil invertebrates (de Bruyn and Conacher, 1990; Goudie, 1988; Lavelle, 1997).

6.3.2.2 Bois Diable Site

At the Bois Diable site a group of large round raised fields was sampled. The site was located close to the western suburbs of the city of Kourou (5°10’31’’ N, 52°39’48’’ W) in a relatively low-lying wet area of a savanna on Pleistocene and Holocene sediments (Boulet, 1975).

The mounds were 4 m in diameter and ca. 60 cm high. The texture in the upper part of the mounds (0 – 20 cm) ranged from silt loam to clay loam and sandy clay loam. In depths > 20 cm it ranged from clay loam to sandy clay loam.

A flat area was sampled close to the mounds. The elevation of this area was intermediate between the elevation of the top of the mounds and the elevation of the ground between the mounds. The flat area had a higher clay content than the mounds in the upper part of the profiles (upper 27 cm). The texture at this depth was clay. Below, sandy clay loam was observed, which provided a comparable texture to the mounds.

6.3.2.3 K-VIII Site

The K-VIII site comprised a complex of large round mounds and elongated ridges. The site was located ca. 5 km northwest of Kourou (5°11’54’’ N, 52°41’12’’ W). As in the Bois Diable site, the raised fields at the K-VIII site were located in a relatively low-lying wet area of a savanna on Pleistocene and Holocene sediments (Boulet, 1975), but the different types of raised fields were arranged over very slightly sloping terrain in a highly organized fashion, as described by McKey et al. (2010) and Rostain (2010). In the upper part of the slope, elongate ridges (ca. 1 – 4 m wide and 25 – 50 m long) were oriented parallel to elevation contours. Down slope, elongate ridges were oriented along the slope. These ridges were similar in width to those found up slope, but were often shorter. Round raised fields with a diameter of ca. 5 m were situated in the flat area at the bottom of the slope. Raised fields at this site were typically ca. 15 – 35 cm high. Some raised fields were ≤ 60 cm high (Rostain, 1991), but these higher raised fields were not sampled.

The texture of the raised fields ranged from silty clay loam to clay loam in the upper 10 cm. Below 10 cm depth, a higher clay content was observed and the texture ranged from silt clay loam to clay.

A flat area was sampled 1.8 km southeast of the raised fields. The elevation of this area was similar to that of the raised fields above the water line (measured in relation to lower-lying wetter areas). However, the flat area had a higher clay content than the raised fields, especially the near-surface horizons of the latter. The texture of soils in the flat area was characterized as clay.

6.3.2.4 Piliwa Site

Large ridges were found at the Piliwa site in northwestern French Guiana (5°43'57" N, 53°53'49" W, Rostain, 2008a). These ridges were located in a small basin on Holocene sediments (Turenne, 1973) close to the sea separated from the estuary of the Mana river only by a sandy ridge. This basin was very wet compared to the other sampled sites. However, there is evidence indicating that the hydrological conditions at the site today differ from those during pre-Columbian times (see Section SI1.3 in Appendix 2.1).

The ridges measured were 20 – 60 m long, 4 – 5 m wide and > 30 cm high. Their texture was heavy clay.

In this very wet, small basin, no flat area was found that was even roughly comparable to the ridges.

6.3.2.5 Organabo Savanna

Medium-sized raised fields were sampled 100 km northwest of Kourou in the Organabo savanna (5°33’14’’N, 53°27’48’’W). The Organabo savanna was located in a shallow wide basin on Pleistocene sediments (Boulet, 1975; Misset, 1967) and was the driest site sampled.

The mounds in this savanna were round or polygonal mounds with flat tops and were densely arranged, separated only by narrow ditches. Their diameter was ca. 3 m and the height of those measured was 16 cm. The texture of the mounds was clay.

A flat area of comparable elevation was sampled close to the mounds. The texture of the flat area was sandier than that of the mounds, ranging from loam to sandy clay.

6.3.2.6 Grand Macoua Savanna

Small raised fields were sampled in the Grand Macoua savanna, 95 km northwest of Kourou (5°32’21’’ N, 53°24’38’’W). Comparable to the Organabo savanna, the Grand Macoua savanna covered a shallow wide basin on Pleistocene sediments (Boulet, 1975; Misset, 1967). Raised fields were found in this savanna in areas which were drier than the sites in Bois-Diable, K-VIII and Piliwa, but slightly wetter than those in the Organabo savanna.

Raised fields in the Grand Macoua savanna varied in their spatial arrangement. In some patches the mounds were densely organised in a square-grid arrangement. In other areas, irregularly arranged mounds were found that were either densely organised or had large flat areas between (scattered arrangement). Inspection of aerial photographs indicated that in the latter areas, many mounds had been obliterated by erosion. The diameter of the mounds which were densely organised in a square-grid arrangement was 50 – 70 cm. The diameter of some irregularly arranged mounds was greater, up to 140 cm. The height of these mounds was ca. 20 cm. The texture of the mounds was silt loam to silty clay loam.

A flat area was sampled close to the mounds with a texture comparable to that of the mounds. This area was located in a lower and wetter part of the savanna than the mounds.

6.3.3 Soil sampling

Each raised field type and each flat area was sampled in five replications. Samples were taken in 10 cm depth increments. For each sample site, at least three depths were chosen for analysis. Depths for analyses were chosen to ensure coverage of both the topsoil horizons and the horizons that were transitional (although still dominated by topsoil material) to the underlying horizons. At least two sampled depths were common to all sites, ensuring comparability: 0 – 10 cm and 20 – 30 cm. Samples were taken from soil profiles or were sampled with an Edelman auger.

6.3.4 Analyses

6.3.4.1 Sample preparation

All samples were dried at 40 °C until they had reached constant weight, and sieved to < 2 mm. Visible roots and other plant parts were removed. This fraction was used for the analysis of pH (KCl) and for extraction of plant-available elements. For the analysis of TOC, total nitrogen and pseudo-total elements, the samples were additionally ground in a bead-mill (MM-2000, Retsch, Hann, Germany).

6.3.4.2 pH measurement

pH was measured in 1 M KCl [1:2.5 (w/v) suspension] using the method described in the 'Procedure for soil analysis' from ISRIC (van Reeuwijk, 2002).

6.3.4.3 Total organic carbon and total nitrogen

TOC and total nitrogen ($N_{tot.}$) were analysed with a CHN-elemental analyser (Thermo Quest, Flash EA, 1112; Thermo Fisher Scientific Inc., Waltham, MA, USA) or with an elemental analyzer (Fisons EA 1108, Fisons Instruments, Milano, Italy) coupled via a Conflow III interface (Finnigan MAT, Bremen, Germany) to an isotope ratio mass spectrometer (Delta S, Finnigan MAT, Bremen Germany).

6.3.4.4 Plant-available element concentrations

Plant-available phosphorus ($P_{avail.}$), calcium ($Ca_{avail.}$), potassium ($K_{avail.}$), magnesium ($Mg_{avail.}$) and sodium ($Na_{avail.}$) were extracted with the Mehlich No. 3 solution (0.2 N acetic acid, 0.25 N ammonium nitrate, 0.015 N ammonium fluoride, 0.013 N nitric acid, and 0.001 M ethylene diamine tetraacetic acid) as described in the 'Soil survey laboratory methods manual' of the NRCS (Burt, 2004). They were measured with an inductively coupled plasma optical emission spectrometer (ICP-OES, Varian Vista-Pro Radial, Varian, Palo Alto, CA, USA).

6.3.4.5 Pseudo-total element concentrations

Pseudo-total phosphorus ($P_{pseudo\text{-}tot.}$), calcium ($Ca_{pseudo\text{-}tot.}$), potassium ($K_{pseudo\text{-}tot.}$), magnesium ($Mg_{pseudo\text{-}tot.}$), copper ($Cu_{pseudo\text{-}tot.}$), barium ($Ba_{pseudo\text{-}tot.}$) and zinc ($Zn_{pseudo\text{-}tot.}$) were extracted with aqua regia as described by Blume et al. (2000). They were measured with an ICP-OES (Varian Vista-Pro Radial, Varian, Palo Alto, CA, USA).

6.3.5 Statistical analysis

Significance of differences of means between different types of raised fields and corresponding flat areas was tested for each site and each depth separately. Homogeneity of variances was tested using the Levene test; deviation from normality was tested using the Shapiro-Wilk test. For data from sites with only one raised field type, significance of differences between raised fields and flat areas was tested using *t*-tests when variance between the groups was homogeneous and values within the groups were normally distributed. The non-parametric Mann-Whitney U-test was used if these conditions were significantly violated. For sites with more than one raised field type, significance of differences was tested by analysis of variance (ANOVA) and Tukey HSD as post-hoc tests, when variance between the groups was homogeneous and values within the groups were normally distributed. In cases where the conditions for ANOVA were significantly violated, non-parametric Kruskal-Wallis tests and Nemeneyi post-hoc tests were used.

To test for relationships between variables, Spearman's rank correlation coefficients were calculated. Correlations were calculated for 0 – 10 cm and 20 – 30 cm depth separately. For the calculation of correlations including only amounts of TOC, $N_{tot.}$ and plant-available nutrients, for both depths, data from all raised fields and flat areas were pooled together. For the calculation of correlations between data, including pseudo-total element concentrations, for both depths, data from raised fields and flat areas at the Bois Diable and K-VIII sites were pooled together (pseudo-total element concentrations were only analysed at these sites; see Section 6.3.4.5). To avoid misinterpretation caused by clusters containing exceedingly high values, we also conducted correlation tests excluding these outlying values. The level of significance was 0.05 for all tests.

6.4 Results

6.4.1 pH

At most sites, the mean pH value was strongly to very strongly acid. With few exceptions, pH value ranged between 3.4 – 4.1 and, consequently, the differences in pH value between raised fields and the associated flat areas were small (Fig. SI2.1 in Appendix 2.2). Only in large ridges at the Piliwa site and in the flat area at the Bois Diable site, pH value > 4.1 was measured (Fig. SI2.1 in Appendix 2.2). In ridges at the Piliwa site, pH value ranged between 4.2 – 4.8, and in the flat area at the Bois Diable site, pH value ranged between 4.1 – 6.2. However, at the Bois Diable site, the differences between mounds and the flat area were not significant.

6.4.2 Total organic carbon and total nitrogen

Moderate to very high amounts of TOC were found in the upper 10 cm of raised fields (Fig. 6.1). The highest TOC content was measured in the upper sampling depths of large ridges at the Piliwa site (Fig. 6.1c). In these ridges, the mean TOC content in the upper 5 cm were close to the concentration defining organic layers.

A significantly higher TOC content of the upper 10 cm of raised fields in comparison to the associated flat area was only found in medium-sized mounds in the Organabo savanna (1.6 ± 0.2 times higher in mounds; Fig. 6.1d). In other raised fields, the TOC content in the upper 10 cm did not differ significantly from that in the associated flat areas.

In all raised fields, the amount of TOC in samples from depths below 10 cm tended to be lower than in the upper 10 cm (Fig. 6.1). Raised fields also tended to have lower TOC content at depths below 10 cm than the upper 10 cm of the associated flat areas (Fig. 6.1). However, at 20 – 30 cm and 40 – 50 cm depths, large mounds in Bois Diable had 6.8 – 36.8 times higher TOC content than the associated flat area (Fig. 6.1a). In the Grand Macoua savanna, at the lower parts of the profiles, the TOC content in small mounds was either higher than in the associated flat area or did not differ significantly between raised fields and the associated flat area (Fig. 6.1e). Thus, at 20 – 30 cm and 30 – 40 cm depths, the small mounds in the Grand Macoua savanna that are densely organised in a square-grid arrangement had ≥ 4.6 times higher TOC content than the flat area in this savanna.

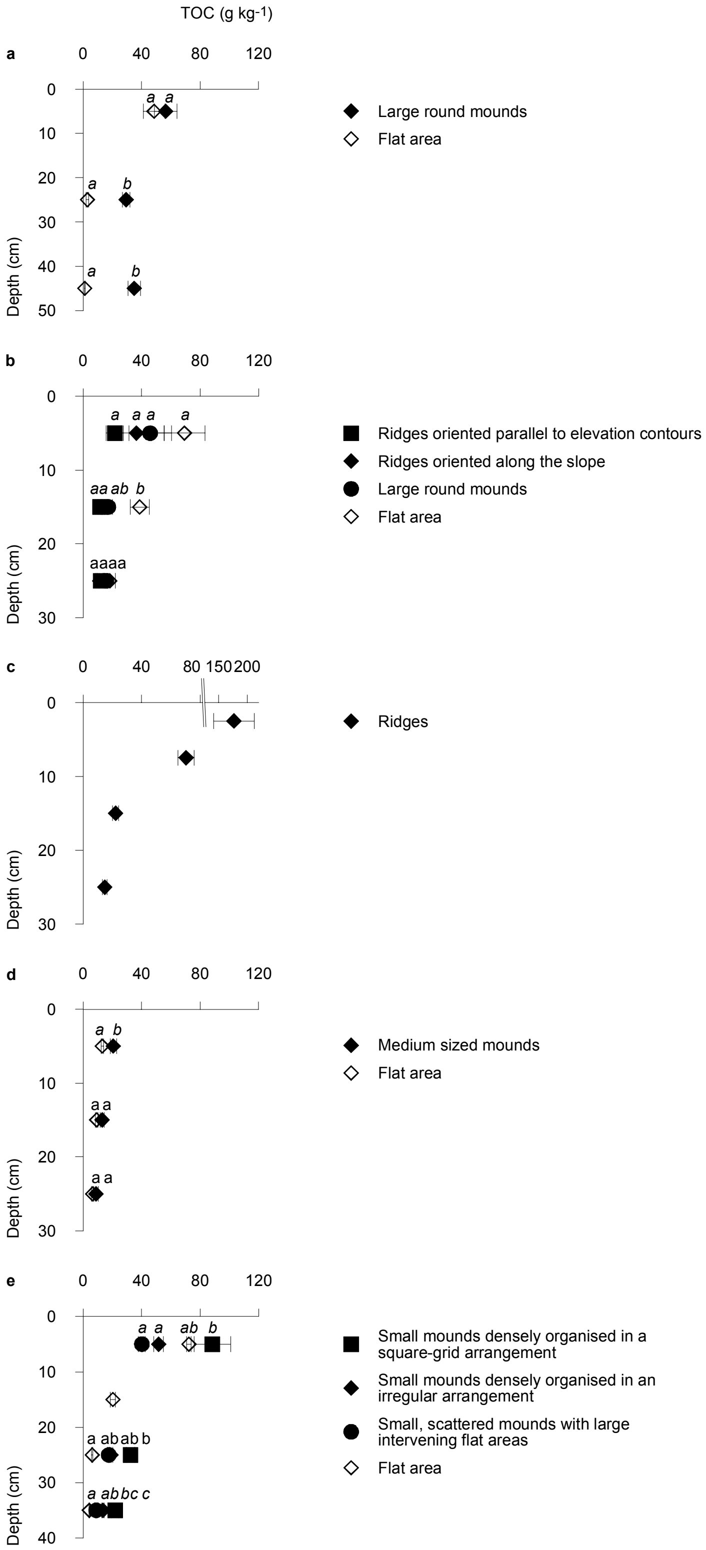

Figure 6.1 Mean amount of total organic carbon (TOC) in raised fields and flat areas. (a) Bois Diable site, (b) K-VIII site, (c) Piliwa site, (d) Organabo savanna, (e) Grand Macoua savanna [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, n = 5].

Additionally, at 30 – 40 cm depth, the small mounds in this savanna that are densely organised in an irregular arrangement had 3.3 ± 0.3 times higher TOC content than the associated flat area.

In contrast to the Bois Diable site and the Grand Macoua savanna, at the K-VIII site, at depths below 10 cm, the amount of TOC in some types of large raised fields were significantly lower than in the associated flat area (Fig. 6.1b). At this site, at 10 – 20 cm depth, the flat area had ≤ 3.7 times higher TOC content than both types of ridges. In ridges at other depths and in round mounds, the TOC content did not significantly differ between raised fields and the associated flat area.

Similar to TOC contents, the mean $N_{tot.}$ content in the upper 10 cm of raised fields was medium to very high (1.2 g kg^{-1} – 9.5 g kg^{-1}; Fig. SI2.2 in Appendix 2.2). The amount of total N was closely related to the TOC content at depths of 0 – 10 cm and 20 – 30 cm ($R \geq 0.95$; $P < 0.05$; Fig. SI2.3 and Fig. SI2.4 in Appendix 2.2).

6.4.3 Plant-available element concentrations

6.4.3.1 Plant-available phosphorus

The concentration of plant-available P was generally low (Fig. 6.2). Concentration of $P_{avail.} > 5.2$ mg kg^{-1} was only found in the upper 10 cm of raised fields at the Bois Diable and Piliwa sites (Fig. 6.2a, c). The relatively high amount in the ridges at the Piliwa site was associated with high TOC concentration (see Section 6.4.2.). However, a correlation of $P_{avail.}$ and TOC was only found for samples at 0 – 10 cm depth and was weak ($R = 0.28$; $P < 0.05$; Fig. SI2.5 and Fig. SI2.6 in Appendix 2.2)

The amount of plant-available P in raised fields either did not significantly differ between raised fields and the flat areas or was slightly higher than in the associated flat areas. At the Bois Diable site, the amount of plant-available P in the upper 10 cm of large mounds was significantly higher than in the flat area, where no plant-available P was measurable (Fig. 6.2a). Significantly higher amount of plant-available P in raised fields than in the associated flat areas was also found in the Organabo savanna at 10 – 20 cm depth, and in the Grand Macoua savanna (Fig. 6.2d, e). In the Grand Macoua savanna, at 20 – 30 cm depth, the small mounds that were densely organized in a square-grid arrangement and the small mounds that were scattered with large intervening flat areas had higher amount of $P_{avail.}$ than the associated flat area. However, differences were small. Enhancement of plant-available P in raised fields in the Organabo and Grand

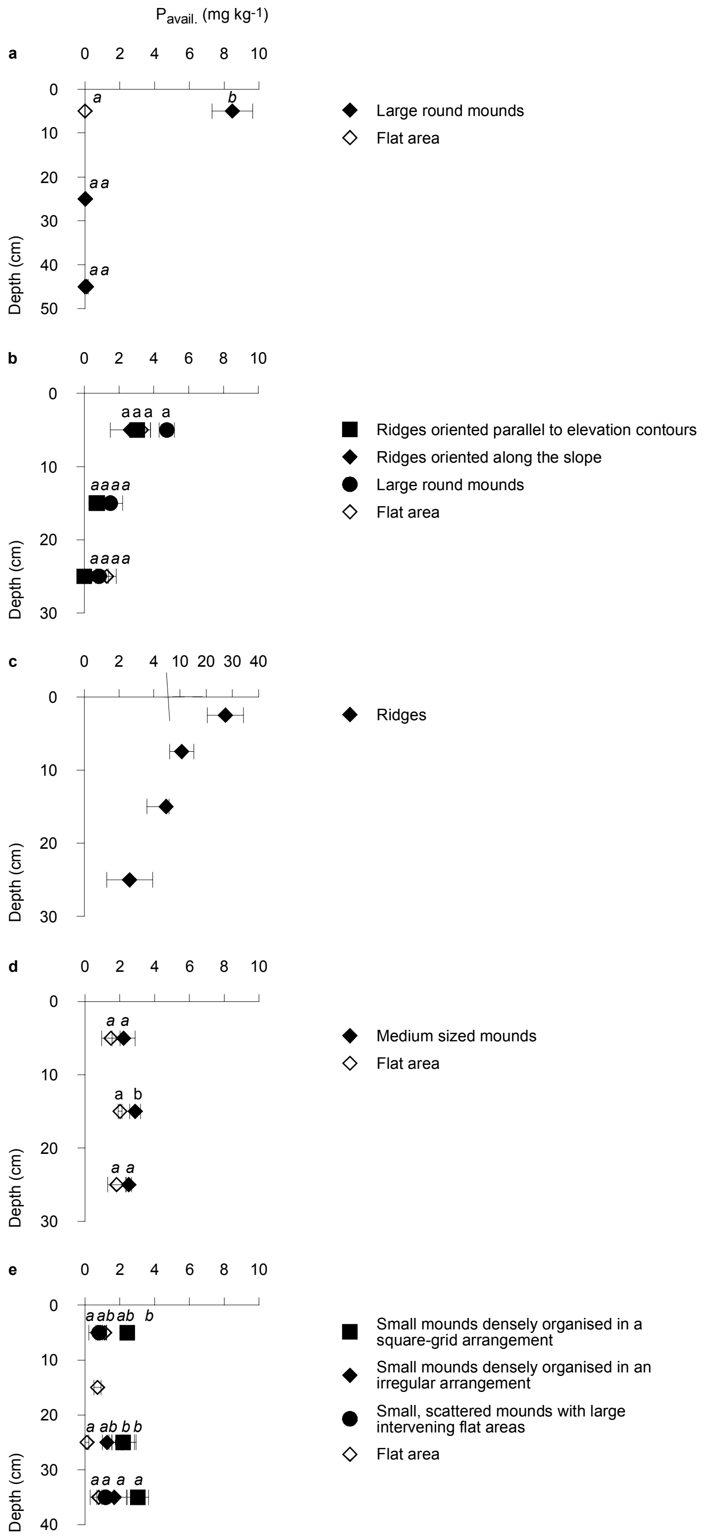

Figure 6.2 Mean plant-available amount of phosphorus ($P_{avail.}$) in raised fields and flat areas. (a) Bois Diable site, (b) K-VIII site, (c) Piliwa site, (d) Organabo savanna, (e) Grand Macoua savanna [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, n = 5].

Macoua savannas compared to the associated flat areas was ≤ 2.8 mg kg^{-1} (Fig. 6.2d, e). Enhancement of plant-available P in large mounds at the Bois Diable site compared to the associated flat area was 8.5 ± 1.2 mg kg^{-1} (Fig. 6.2a).

6.4.3.2 Plant-available Ca, K, Mg and Na

In the ridges at the Piliwa site, the amounts of plant-available K, Mg and Na were high and the amount of plant-available Ca was moderate to low (Fig. 6.3c). In all other raised fields, the concentrations of plant-available Ca and K were low and the amount of plant-available Na was not exceedingly high (Fig. 6.3a, b, d, e). The amount of plant-available Mg was low in medium-sized mounds in the Organabo savanna (Fig. 6.3d) and low to moderate in raised fields at the Bois Diable and K-VIII sites and in the Grand Macoua savanna (Fig. 6.3a, b, e).

The concentrations of plant-available Mg, Ca and Na were positively correlated in the upper 10 cm ($R \geq 0.73$; $P < 0.05$; Fig. SI2.7a in Appendix 2.2). If a single cluster with relatively high values (see Section 6.4.5) is removed, the amount of $K_{avail.}$ (< 200 mg kg^{-1}) was also correlated with the amounts of $Ca_{avail.}$ (< 200 mg kg^{-1}), $Mg_{avail.}$(< 400 mg kg^{-1}), and Na_{avail} (< 150 mg kg^{-1}) in the upper 10 cm ($R \leq 0.59$; $P < 0.05$; Fig. SI2.7b in Appendix 2.2). At 20 – 30 cm depth, the values for the clusters of relatively low amounts of $Ca_{avail.}$, $K_{avail.}$, $Mg_{avail.}$ and Na_{avail}. all showed significant positive pairwise correlations ($R \geq 0.54$; $P < 0.05$; Fig. SI2.8b in Appendix 2.2).

Only in the Organabo and in the Grand Macoua savannas, amounts of some plant-available cations were enhanced in a few raised fields, at some depths, compared to the flat areas. Medium-sized mounds in the Organabo savanna had 1.6 ± 0.2 times higher plant-available K concentration at depths below 10 cm compared to the flat area in this savanna (Fig. 6.3d); and in the Grand Macoua savanna, 2.2 ± 0.6 times higher amount of plant-available Ca was found in the upper 10 cm of the small, scattered round mounds with large intervening flat areas, compared to the associated flat area (Fig. 6.3e).

In the Organabo savanna, the amount of $K_{avail.}$ at depth 0 – 10 cm and the amounts of the other plant-available cations at all depths, did not differ significantly between mounds and the flat area (Fig. 6.3d). In the Grand Macoua savanna, apart from the scattered mounds with large intervening flat areas, $Ca_{avail.}$ was not enriched in mounds compared to the flat area. In contrast, the densely organised mounds arranged in a square grid had significantly lower amount of $Ca_{avail.}$ at depths below 10 cm than in the flat area (≤ 2.3 times higher in the flat area; Fig. 6.3e). In this savanna, frequently $Mg_{avail.}$ and especially

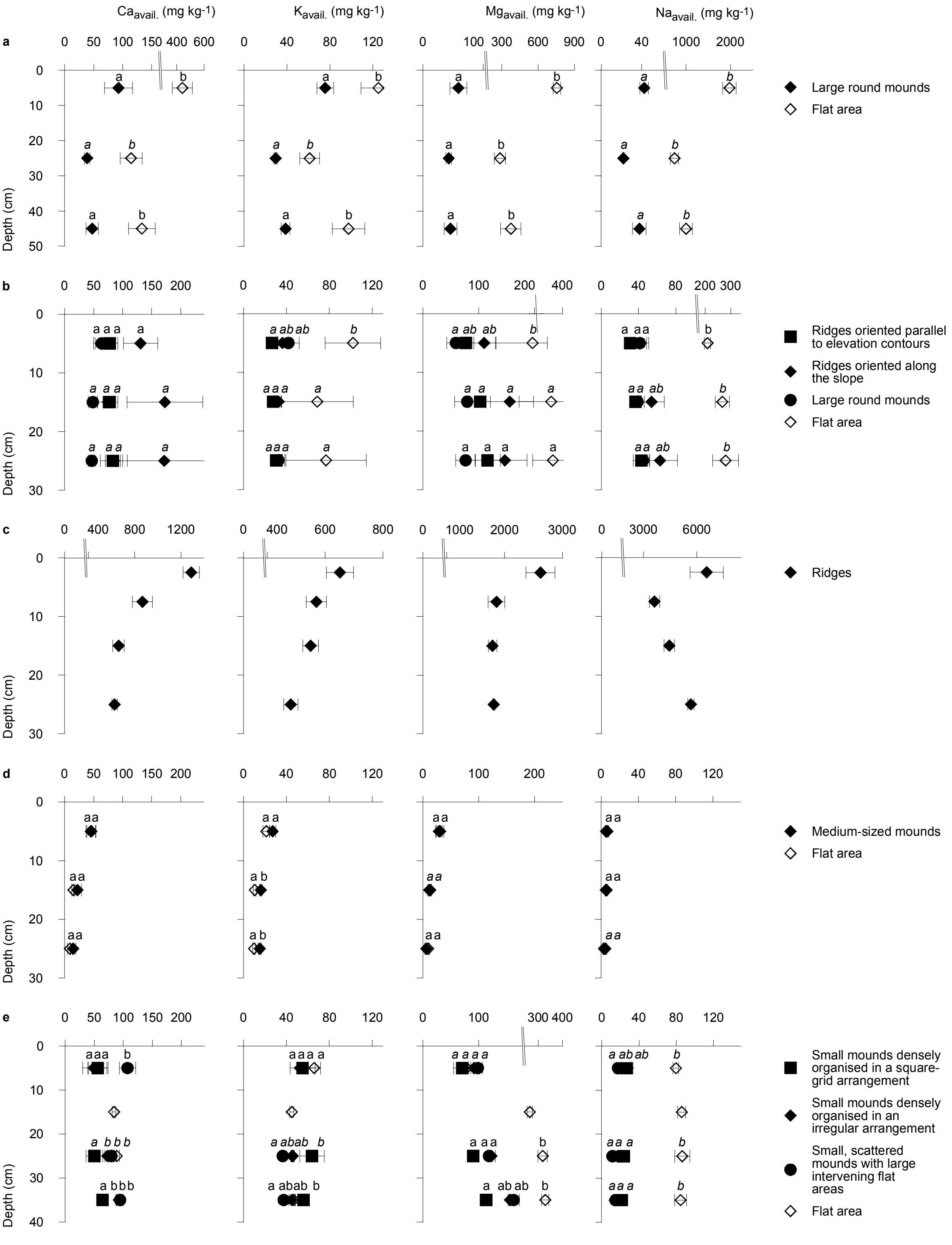

Figure 6.3 Mean plant-available amounts of calcium ($Ca_{avail.}$), potassium ($K_{avail.}$), magnesium ($Mg_{avail.}$) and sodium ($Na_{avail.}$) in raised fields and flat areas. (a) Bois Diable site, (b) K-VIII site, (c) Piliwa site, (d) Organabo savanna, (e) Grand Macoua savanna [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, n = 5].

$Na_{avail.}$ were present at significantly lower concentrations in mounds than in the associated flat area. For all mounds, at depths below 10 cm, the amount of $Na_{avail.}$ was 3.0 – 8.6 times higher in the flat area than in mounds (Fig. 6.3e). A comparable decrease of $Na_{avail.}$ in the upper 10 cm of mounds compared to the flat area was also found in scattered mounds with large intervening flat areas (Fig. 6.3e). At 20 – 30 cm depth in this site, for all mound types, the amount of $Mg_{avail.}$ in the flat area was 2.4 – 4.0 times higher than in mounds (Fig. 6.3e). Additionally, at 30 – 40 cm depth in densely organised mounds arranged in a square grid, the amount of $Mg_{avail.}$ was 2.9 ± 0.3 times lower than in the corresponding flat area (Fig. 6.3e). In other samples from in the Grand Macoua savanna, no significant differences were found in amounts of plant-available Mg and Na between mounds and the flat area (Fig. 6.3e). In all types of mounds, the amount of $K_{avail.}$ did not differ significantly in comparison to flat areas (Fig. 6.3e).

In large raised fields at the Bois Diable and K-VIII sites, amounts of plant-available cations were frequently lower than in the associated flat areas. As found for the Grand Macoua savanna, differences were particularly marked for plant-available Na at these sites. In mounds at the Bois Diable site, amounts of all analysed plant-available cations were significantly lower at all depths than in the flat area (Fig. 6.3a). Among the flat areas of all sites examined, the flat area at the Bois Diable site showed the highest amounts of these elements. The relative enhancement of the concentrations in the flat area relative to the mounds was higher for $Na_{avail.}$ ($\geq$ 20.4 times) and $Mg_{avail.}$ (5.4 – 16.0 times) than for $Ca_{avail.}$ (2.0 - 6.2 times) and $K_{avail.}$ ($\leq$ 2.4 times). Also, the difference in the concentrations between the flat area and the mounds was $Na_{avail.} > Mg_{avail.} > Ca_{avail.} > K_{avail.}$ (each depth compared separately; Fig. 6.3a).

At the K-VIII site, the concentration of $Na_{avail.}$ was 3.9 – 7.8 times higher in the upper 10 cm of the flat area than in all types of raised fields and 4.7 – 8.7 times higher in the flat area at 20 – 40 cm depth than in ridges arranged parallel to the elevation contours and in round mounds (Fig. 6.3b). In the upper 10 cm of the flat area, the amount of K_{avail} was 3.8 ± 1.2 times higher than in the ridges arranged parallel to the elevation contours, and the amount of $Mg_{avail.}$ was 3.3 ± 1.5 times higher than in round mounds (Fig. 6.3b).

6.4.4 Pseudo-total nutrient amounts

(large raised fields at the Bois Diable and the K-VIII sites)

6.4.4.1 Pseudo-total phosphorus

The amount of pseudo-total P in raised fields and flat areas at the Bois Diable and K-VIII sites (Fig. 6.4) was several times higher than the amount of plant-available P (Fig. 6.2a, b). No correlation between pseudo-total P and plant-available P was found at either 0 – 10 cm or 20 – 30 cm depth (Fig. SI2.9 and Fig. SI2.10 in Appendix 2.2).

The amount of $P_{pseudo\text{-}tot.}$ was closely correlated with the amount of TOC in the upper 10 cm ($R = 0.92$; $P < 0.05$; Fig. SI2.11 in Appendix 2.2). Amounts of $P_{pseudo\text{-}tot.}$ and of TOC were also found to be correlated at 20 - 30 cm depth ($R = 0.64$; $P < 0.05$; Fig. SI2.12 in Appendix 2.2). As found for TOC content, the amount of $P_{pseudo\text{-}tot.}$ was significantly higher in mounds than in the flat area at the Bois Diable site at depths of 20 – 30 cm and 40 – 50 cm and did not differ in the upper 10 cm (Fig. 6.4a). The differences of the $P_{pseudo\text{-}tot.}$ concentration between raised fields at the K-VIII site and the associated flat area were not completely coincident with the differences of TOC, and lower amount of $P_{pseudo\text{-}tot.}$ in raised fields compared to the flat area was predominantly found in ridges (Fig. 6.4b).

6.4.4.2 Pseudo-total calcium, potassium and magnesium

The amount of $Ca_{pseudo\text{-}tot.}$ in raised fields and flat areas at the Bois Diable and K-VIII sites (Fig. 6.4) was not much higher than the amount of plant-available Ca (Fig. 6.3a, b) and the concentration of pseudo-total Ca was closely correlated with the amount of plant-available Ca at depths of 0 – 10 cm ($R = 0.94$; $P < 0.05$; Fig. SI2.9 in Appendix 2.2) and 20 – 30 cm ($R = 0.82$; $P < 0.05$; Fig. SI2.10 in Appendix 2.2).

As found for the concentration of $Ca_{avail.}$, no significant difference in the amount of $Ca_{pseudo\text{-}tot.}$ was found between raised fields and the associated flat area at the K-VIII site (Fig. 6.4b). Comparable to the amount of plant-available Ca, the amount of pseudo-total Ca was 1.5 – 4.4 times lower in mounds than in the associated flat area at the Bois Diable site (Fig. 6.4a). Also, difference in the amount of pseudo-total Ca between the mound and the flat area at this site (Fig. 6.4a) was comparable to the difference in the concentration of plant-available Ca (Fig. 6.3a).

The amounts of pseudo-total K and Mg in raised fields at the Bois Diable and K-VIII sites (Fig. 6.4) were several times higher than the amounts of plant-available K and Mg (Fig. 6.3a, b). the pseudo-total amounts of these two elements were closely correlated at depths of 0 – 10 cm ($R = 0.84$; $P < 0.05$; Fig. SI2.13 in Appendix 2.2) and 20 – 30 cm ($R = 0.96$; $P < 0.05$; Fig. SI2.14 in Appendix 2.2). The plant-available and pseudo-total amounts of K were not correlated (Fig. SI2.11 and Fig. SI2.12 in Appendix 2.2), but the amounts of $Mg_{pseudo\text{-}tot.}$ and $Mg_{avail.}$ were correlated ($R \leq 0.85$; $P < 0.05$; Fig. SI2.11 and Fig. SI2.12 in Appendix 2.2). The correlation coefficients between $Mg_{pseudo\text{-}tot}$ and $Ca_{pseudo\text{-}tot.}$ were ≤ 0.52 ($P < 0.05$; Fig. SI2.13 and Fig. SI2.14 in Appendix 2.2). No correlation was found between the amounts of $K_{pseudo\text{-}tot.}$ and of $Ca_{pseudo\text{-}tot.}$ (Fig. SI2.13 and Fig. SI2.14 in Appendix 2.2).

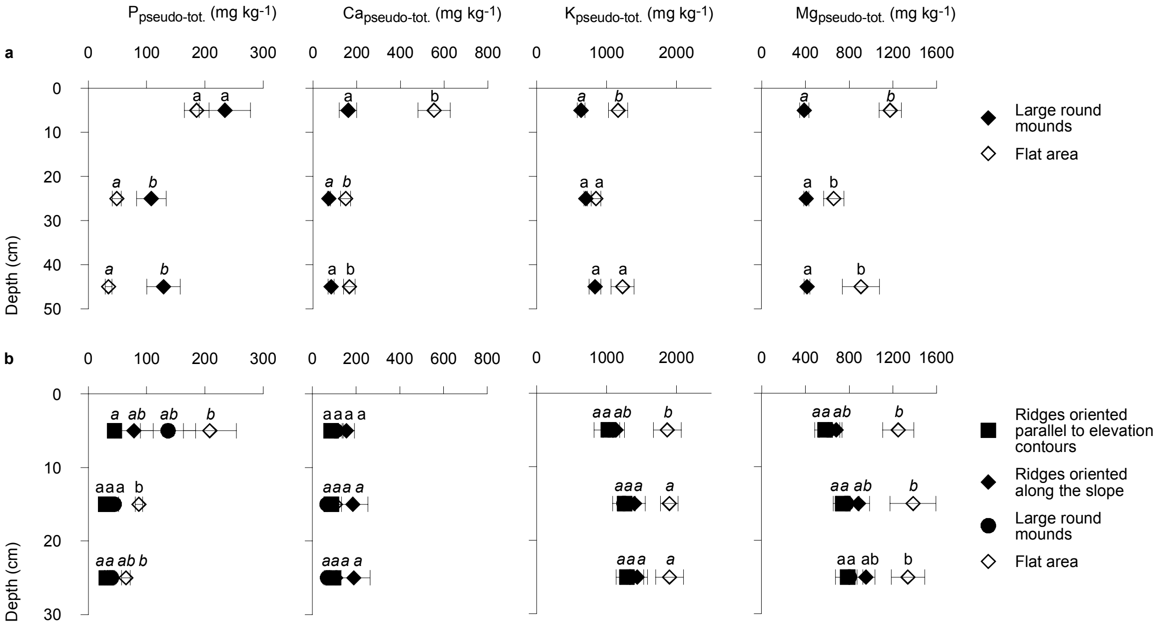

Figure 6.4 Mean pseudo-total amounts of phosphorus ($P_{pseudo\text{-}tot.}$), calcium ($Ca_{pseudo\text{-}tot.}$), potassium ($K_{pseudo\text{-}tot.}$) and magnesium ($Mg_{pseudo\text{-}tot.}$), in raised fields and flat areas. (a) Bois Diable site and (b) K-VIII site [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, $n = 5$].

The amount of pseudo-total Mg was lower in mounds at the Bois Diable site, and in ridges oriented parallel to the elevation contours and round mounds at the K-VIII site, than in the associated flat areas (Fig. 6.4). At both sites, differences were particularly marked in the upper 10 cm. At the Bois Diable site, differences in the amount of pseudo-total Mg (Fig. 6.4a) were comparable to the differences in the amount of plant-available Mg (Fig. 6.3a). In contrast, at the K-VIII site, the differences in the amount of pseudo-total Mg (Fig. 6.4b) were more pronounced than the differences in the amount of plant-available Mg (Fig. 6.3b).

As found for pseudo-total Mg, the amount of pseudo-total K was lower in the upper 10 cm of mounds at the Bois Diable site, and of ridges oriented parallel to the elevation contours and round mounds at the K-VIII site, than in the associated flat areas (Fig. 6.4). However, at both sites, differences in the amount of pseudo-total K were more pronounced than the differences in the amount of plant-available K in the upper 10 cm (Fig. 6.3a, b and Fig. 6.4). In depths below 10 cm, the amount of $K_{\text{pseudo-tot.}}$ did not significantly differ between mounds and the associated flat areas (Fig. 6.4).

6.4.4.3 Pseudo-total copper, barium and zinc

The amounts of pseudo-total Cu, Ba and Zn were not high in raised fields at Bois Diable and K-VIII sites (Fig. 6.5). At 0 – 10 cm and 30 – 30 cm depth, values of these elements were positively correlated with each other ($R \geq 0.59$; $P < 0.05$; Fig. SI2.13 and Fig. SI2.14 in Appendix 2.2), with the amounts of $K_{\text{pseudo-tot.}}$ and $Mg_{\text{pseudo-tot.}}$ ($R \geq 0.53$; $P < 0.05$; Fig. SI2.13 and Fig. SI2.14 in Appendix 2.2) and with the amount of TOC ($R \geq 0.39$; $P < 0.05$; Fig. SI2.11 and Fig. SI2.12 in Appendix 2.2). In the upper 10 cm, the concentration of $Zn_{\text{pseudo-tot.}}$ was correlated with the amount of $P_{\text{pseudo-tot.}}$ ($R = 0.39$; $P < 0.05$; Fig. SI2.13 in Appendix 2.2).

At the Bois Diable site, comparable to the amounts of $K_{\text{pseudo-tot.}}$ and $Mg_{\text{pseudo-tot.}}$ (see Section 6.4.4.2), the amount of $Zn_{\text{pseudo-tot.}}$ was lower in mounds than in the flat area in the upper 10 cm (Fig. 6.5a). Comparable to the TOC concentration (see Section 6.4.2), the amounts of $Ba_{\text{pseudo-tot.}}$ and $Cu_{\text{pseudo-tot.}}$ were higher in mounds than in the flat area at depths below 10 cm at this site (Fig. 6.5a). Also, $Zn_{\text{pseudo-tot.}}$ was higher concentrated in mounds than in the flat area at depths of 40 – 50 cm (Fig. 6.5a) at this site.

In raised fields at the K-VIII site, roughly parallel to variation in the concentration of TOC and the amounts of pseudo-total K and Mg, the amounts of $Cu_{\text{pseudo-tot.}}$, $Ba_{\text{pseudo-tot.}}$ and $Zn_{\text{pseudo-tot.}}$ were either lower compared to the associated flat area, or values for these two soil types were not significantly different (Fig. 6.5b).

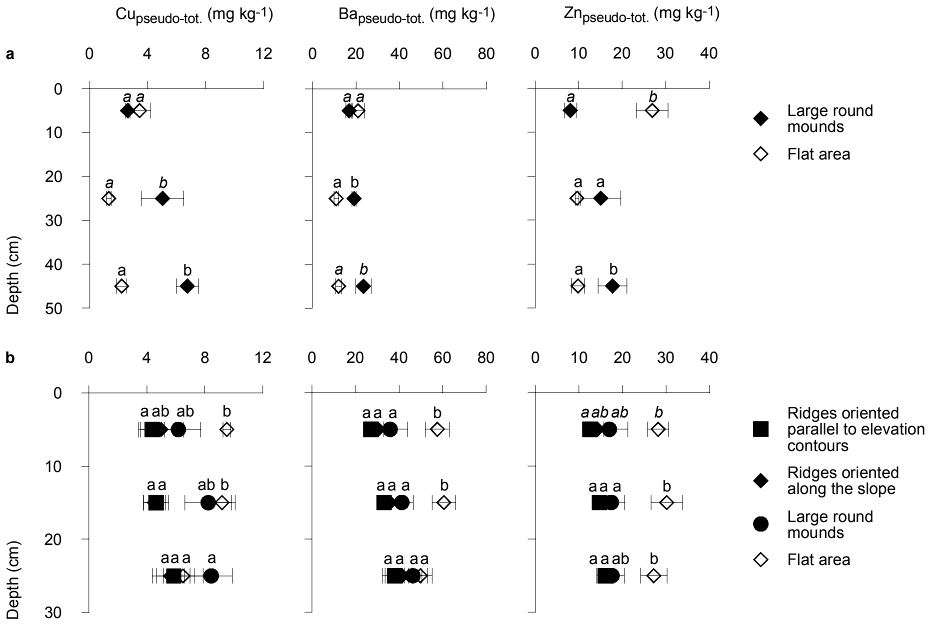

Figure 6.5 Mean pseudo-total amounts of copper ($Cu_{pseudo\text{-}tot.}$), barium ($Ba_{pseudo\text{-}tot.}$) and zinc ($Zn_{pseudo\text{-}tot.}$), in raised fields and flat areas. (a) Bois Diable site and (b) K-VIII site [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, n = 5].

6.5 Discussion

6.5.1 Total organic carbon and application of organic material

The higher TOC concentration in the upper 10 cm of medium-sized mounds in the Organabo savanna compared to the associated flat area could be caused by the higher clay content in these mounds compared to the flat area (see Section 6.3.2.5) (Feller and Beare, 1997; Zech et al., 1997). However, the enhanced concentration of TOC in raised fields at the Bois Diable site and in the Grand Macoua savanna was not accompanied by higher clay content in raised fields compared to the associated flat areas (see Sections 6.3.2.2 and 6.3.2.6).

Based on the abundance over time of charcoal particles in a peat core close to the K-VIII site, Iriarte et al. (2012) concluded that pre-Columbian raised-field agriculture in French Guiana was largely fire-free. Renard et al. (2012b) speculated that slashed biomass of fallow vegetation and biomass and litter from the basins between raised fields may have been applied onto raised fields. They argued that especially in very wet basins, litter accumulates from algae and aquatic macrophytes with low C:N and C:P ratios.

Consequently, they hypothesised that plant biomass and litter from basins could have been an important source of nutrients in raised fields in very wet areas where the basins between raised fields are water-saturated for long periods. Furthermore, crop residues are virtually incorporated universally into contemporary analogues of raised fields outside of Amazonia (Crews and Gliessman, 1991; Denevan and Turner, 1974; Domingo and Hagerman, 1982; Kirch, 1978; Kleinhenz, 1997). Renard et al. (2012b) interpreted phytoliths of vegetative parts of maize, found in raised fields in French Guiana (Iriarte et al., 2010), as indications of mulching with crop residues. Thus, the enhanced amount of TOC in raised fields compared to associated flat areas in the Grand Macoua savannas and in the Bois Diable site could have originated from addition of slashed plant biomass and litter onto raised fields by pre-Columbian farmers. Such management practices could also have contributed to the enhanced amount of TOC in mounds in the Organabo savanna in comparison to the flat area in this savanna, in addition to the effects of a difference in clay content between mounds and the associated flat area.

The TOC concentration in raised fields in the Grand Macoua savanna and the Bois Diable site was higher than in the associated flat areas only at depths below 10 cm. At these depths, the TOC concentration was lower than in the upper 10 cm of mounds and the associated flat areas. Such an elevation of the amount of TOC could have been simply caused by the addition of topsoil material onto raised fields during their construction. Additionally, transfer of sediments in combination with vegetation from the basins between raised fields to the cropping area could have caused this elevation of TOC concentration. Such transfer is an inevitable consequence of maintaining raised fields, and is universal in extant analogous systems outside of Amazonia (Crews and Gliessman, 1991; Denevan, 2001; Vasey et al., 1984). However, it is hard to distinguish the addition of slashed plant material and litter from the transfer of basin sediments in combination with plant material to raised fields during their construction and subsequent maintenance.

In addition to the direct movement of material with high carbon content by pre-Columbian farmers, soil invertebrates could also have contributed to higher TOC concentration in mounds compared to the associated flat areas. McKey et al. (2010) described the transport of organic matter by ants and termites to their nests in raised fields. An increase of organic matter in ant nests has frequently been reported (Frouz and Jilková, 2008). Analyses of soil invertebrates in small raised fields showed that termites of the subfamily Nasutitermitinae are the most abundant termites in these raised fields (McKey et al., 2010; Renard et al., 2013). Higher carbon content in termite mounds than in surrounding soils has been found in nests of various mound-building termites, including Nasutitermitinae, e.g. in savannas in Colombia (Decaëns et al., 2001), in secondary forests in central Amazonia (Ackerman et al., 2007) and in pastures in Minas Gerais, Brazil (Sarcinelli et al., 2009). Additionally, earthworms belonging to the genus *Martiodrilus* were found in small raised fields in Grand Macoua (Renard et al., 2013). Studies in the Eastern Plains of Colombia showed that carbon is stabilized in the casts of *Martiodrilus* earthworms (Guggenberger et al., 1996; Mariani et al., 2007). Thus, natural ecosystem engineers could also have contributed to enhanced TOC content in raised fields.

For the K-VIII site, McKey et al. (2010) and Iriarte et al. (2010) found signs that raised fields were not built by accumulation of topsoil material within the immediate area where the raised fields were found, but with material mainly transported from a higher, less flooded area < 100 m from the raised fields. Topsoil in the higher area may have had lower SOM contents than the topsoil in the wetter area. This could explain that no elevated amount of TOC was found in large raised fields at the K-VIII site. Additionally, these raised fields had lower clay content than the associated flat area, and this could have contributed to lower TOC content in ridges compared to the flat area at 10 – 20 cm depth.

The raised fields at the Piliwa site are located in a very low-lying, wet area (see Section 6.3.2.4) and covered by Cyperaceae (mainly *Eleocharis* sp.) Under these conditions, accumulation of organic matter at the surface is typical in the coastal area in French Guiana (Leprun et al., 2001). Thus, the high TOC content concentrated in the upper cm of ridges at Piliwa can be explained by the hydrological conditions at the site today, which likely differ from those during pre-Columbian times (see Section SI1.3 in Appendix 2.1).

The close correlation between $N_{tot.}$ and TOC, showed that the concentration of $N_{tot.}$ was mainly determined by the amount of SOM.

6.5.2 Amounts of phosphorus and fertilization

The enhanced concentration of plant-available P in raised fields did not correlate with higher pH value in raised fields compared to the associated flat areas at the Bois Diable site and in the Organabo and Grand Macoua savannas. Only in the Organabo savannah, enhanced amount of plant-available P correlated with higher clay content in mounds (see Section 6.3.2.5).

As plant-available P is a classical indicator for human deposition of nutrient-rich materials (Bethel and Máté, 1989; Holliday and Gartner, 2007), these data could indicate fertilization with material with high P content by pre-Columbian farmers. However, as summarized by Burt (2004) and Ziadi and Tran (2008), the extraction with Mehlich No. 3, combined with ICP-OES measurements—both techniques were used in this study—can yield higher amount of P than extractions with other widely used solutions for the determination of plant-available P and colorimetric methods determining only orthophosphate. Although higher amount of plant-available P would be expected by the analysis used in this study compared to other analyses of plant-available P, the amount of plant-available P in raised fields was low and the differences between raised fields and associated flat areas were small. This shows that pre-Columbian agriculture on raised fields in French Guiana did not lead to considerably higher amount of plant-available P in soils today. Additionally, the analysis of plant-available and pseudo-total P at the Bois Diable site indicated that even the largest difference in plant-available P found between raised fields and associated flat areas in this study was not caused by total P enrichment on mounds. The enhanced amount of plant-available P in the uppermost 10 cm of these large mounds compared to the flat area at this site was not associated with higher amount of $P_{\text{pseudo-tot.}}$ in mounds at this depth. Pseudo-total P was enhanced in these mounds only at depths below 10 cm in relation to the associated flat area. The relative enhancement of $P_{\text{pseudo-tot.}}$ in mounds was smaller than the enhancement of TOC in mounds in relation to the flat area. Additionally, the correlation between the amounts of pseudo-total P and TOC at the Bois Diable and K-VIII sites indicates that the differences in the amount of pseudo-total P between raised fields and flat areas were caused by different concentration of SOM and were not caused by addition of especially P-rich materials.

It is a matter of debate whether easily extractable P, or total and pseudo-total P, is more sensitive for detecting human soil modification. On the one hand, as found for other elements, human-induced changes in the concentrations of easily extractable P fractions have been considered to persist in soil over shorter time periods compared to changes in the pseudo-total and total concentrations. On the other hand, differences caused by human soil modifications in the easily extractable fraction could be overwhelmed by much higher natural background concentrations obtained by pseudo-total and total extractions (Holliday and Gartner, 2007; Middleton, 2004; Middleton and Price, 1996; Wilson et al., 2006). While the small enhancement in the concentration of plant-available P could conceivably be indicative of fertilization of raised fields with materials with high P content, this enhancement could also be due to the activities of natural ecosystem engineers inhabiting the raised fields. The activity of soil macrofauna generally enhances the availability of P (Seastedt, 1995). The availability of P is usually higher in earthworm casts and ant nests than in soils without these structures (Chapuis-Lardy et al., 2011; Frouz and Jilková, 2008). Phosphorus availability is often enhanced in termite mounds, but data are sometimes contradictory and materials used by termites in feeding and nest-building have a strong influence on P availability (Chapuis-Lardy et al., 2011; Decaëns et al., 2001; López-Hernández et al., 2006; Rückamp et al., 2010; Sarcinelli et al., 2009). A number of studies have reported that enhanced availability of P in biogenic structures is correlated with more neutral pH values (Frouz and Jilková, 2008; Sarcinelli et al., 2009). However, earthworms of the family Glossocolecidae, *Pontoscolex corethrurus* Müller and *Martiodrilus* sp., were the most frequently found species in small raised fields in Grand Macoua (Renard et al., 2013). In a rainforest Oxisol in Brazil, higher P availability in the casts of Glossoscolecidae was not associated with higher pH values (Kuczak et al., 2006). Specifically, enhanced P availability in the casts of the earthworm *Pontoscolex corethrurus* Müller is assumed not to be associated with changes of pH in the cast (Lopez-Hernandez, 1993).

Today, the savannas that harbour our sites are frequently burned. Lavelle (1978, in Lavelle and Spain 2001) showed that anecic earthworms incorporate less litter in regularly burned savannas in Ivory Coast than in less burned savannas. Furthermore, Sousa-Souto et al. (2008) showed that enhancement of plant-available P in the nests of leaf-cutting ants in Brazilian savannas (*cerrado*) is diminished by regular burning. Accordingly, effects of soil invertebrates could have been more pronounced during the fire-free agricultural use of raised fields in French Guiana (see Section 6.5.1) postulated by Iriarte et al. (2012).

Although only a weak correlation was found between TOC and the amount of plant-available P at 0 – 10 cm depth, the relatively high amounts of $P_{avail.}$ in raised fields at the Piliwa site could be caused simply by the high amount of SOM in the upper cm of these raised fields, combined with slightly higher pH value and lower content of Fe(III) oxides than in other sites (see Section SI1.3 in Appendix 2.1). Compared to reported data from another soil with an organic layer in the coastal area of French Guiana (Blancaneaux, 1981), the amount of $P_{avail.}$ in the Piliwa ridges does not indicate an extraordinary enrichment of plant-available P in these raised fields.

6.5.3 Concentrations of Ca, K, Mg and Na

6.5.3.1 Influences of clay content on concentrations of Ca, K, Mg and Na

Higher clay content was observed in the flat areas compared to raised fields at the Bois Diable and K-VIII sites. The clay content differed particularly in the upper parts of the profiles, but also differed slightly in the lower parts of the profiles at the K-VIII site (see Sections 6.3.2.2 and 6.3.2.3). The lower amount of pseudo-total K in the upper 10 cm of large mounds at the Bois Diable site and some types of large raised fields at the K-VIII site compared to the corresponding flat areas likely reflect higher amounts of illite and other three-layer clay minerals (see Section 6.3.1) in these flat areas (see Sections 6.3.2.2 and 6.3.2.3). At these sites, the amounts of pseudo-total Mg and K were correlated. Thus the higher clay content in the flat areas at these sites could contribute to the higher amount of pseudo-total Mg in the flat areas at these depths compared to the mounds at the Bois Diable site and some types of raised fields at the K-VIII site. These differences in the clay content could also contribute, at the Bois Diable site, to the higher amounts of pseudo-total Ca and plant-available cations in the upper part of the flat area compared to mounds and at the K-VIII site, and to the higher amounts of plant-available K, Mg and Na in some depths of the flat area compared to some types of raised fields.

The enhanced amount of plant-available K in medium-sized mounds in the Organabo savanna compared to the associated flat area was correlated with a higher clay content in mounds compared to the associated flat area (see Section 6.3.2.5; pseudo-total element concentrations were not analysed in the Organabo savanna). The texture of mounds differed considerably from that of the associated flat area in this savanna. However, in contrast to the Bois Diable and K-VIII sites, the sediments in the Organabo savanna were deposited during the Pleistocene containing only low amounts of three-layer clay minerals (see Section 6.3.1). The low amount of three-layer clay minerals in this savanna likely explain why the amount of plant-available K was only slightly enhanced in mounds in comparison to the associated flat area and why no significant differences of plant-available Ca, Mg and Na were found between mounds and the flat area.

6.5.3.2 Influence of brackish groundwater on concentrations of Ca, K, Mg and Na

The lower amounts of Ca, K, Mg and Na in large mounds at the Bois Diable site at 30 – 40 cm depth compared to the associated flat area were not accompanied by differences in clay contents (see Section 6.3.2.2). Similarly, the higher amounts of plant-available

Mg and Na in the flat area in the Grand Macoua savanna compared to small mounds at this site were also not accompanied by differences in clay contents (see Section 6.3.2.6).

However, brackish groundwater was observed in the coastal savannas in French Guiana (see Section 6.3.1). Differences in the amounts of plant-available Na and Mg at the Bois Diable site and in the Grand Macoua savanna were more pronounced than differences in the amounts of other plant-available cations between raised fields and the associated flat areas. Also, at the K-VIII site, differences in the amount of plant-available Na were more pronounced than differences in the amounts of other plant-available cations between raised fields and the associated flat area. Thus, the relatively high amounts of plant-available Mg, and especially plant-available Na, in the flat areas in comparison to raised fields most likely indicate a more pronounced influence of brackish groundwater on the flat areas than on raised fields at the Bois Diable and K-VIII sites and in the Grand Macoua savanna. The positive pairwise correlations between the amounts of $Ca_{avail.}$, $K_{avail.}$, $Mg_{avail.}$ and $Na_{avail.}$ show that amounts of the plant-available fraction of all analysed cations were likely influenced by brackish groundwater at these sites.

Pseudo-total Mg and plant-available Mg were correlated. Furthermore, the differences in concentrations between large mounds and the flat area at the Bois Diable site were comparable for the pseudo-total and the plant-available fractions of Mg. Thus, a more pronounced influence of brackish groundwater on the flat area than on mounds may have caused the differences in the amount of pseudo-total Mg at the Bois Diable site at depths > 27 cm. Brackish groundwater also likely affected the amount in the upper parts of the profiles, in addition to the effects of differing clay contents at this depth.

Pseudo-total Ca was correlated with pseudo-total Mg. The correlation between the plant-available and pseudo-total fractions of Ca, and their comparable concentration ranges, both show that the Ca extracted by the pseudo-total extraction was dominated by plant-available Ca. As found for the concentrations of Mg, at the Bois Diable site the differences in concentrations between mounds and the flat area were comparable for the pseudo-total and the plant-available fractions of Ca, indicating an influence of brackish water on the pseudo-total concentrations of this element.

In the Grand Macoua savanna, the slightly different topographic position of raised fields and the flat area (see Section 6.3.2.6) could cause the apparent greater influence of brackish groundwater on the flat areas compared to the raised fields. At the Bois Diable and K-VIII sites, the topographic position of raised fields and associated flat areas were comparable (see Sections 6.3.2.2 and 6.3.2.3). Construction of large raised fields at these sites seems to have promoted a leaching of salt under specific conditions of high precipitation and seasonal flooding with freshwater. However, analyses of samples collected during different seasons would be necessary to investigate if this effect persists during all seasons.

Whether brackish groundwater was an important ecological factor when raised fields in French Guiana were being cultivated in pre-Columbian times is not yet clear. Misset (1967) interpreted enhanced salt contents in the coastal savannas in French Guiana as a result of seawater intrusion within the preceding decade. In contrast, Boulet (1975) explained the occurrence of brackish groundwater in these savannas by low permeability of the sediments, inhibiting drainage of the salt water in areas separated from the sea for long periods, similar to observations in a comparable area in Brazil (central part of Marajó island; Sombroek, 1963). Whereas at the Bois Diable site significantly lower amounts of plant-available Ca, K, Mg and Na and pseudo-total Ca and Mg were found in raised fields compared to the associated flat area, the differences were less clear for all of these elements at the K-VIII site. At this site, a smaller influence of brackish groundwater in the flat area compared to the Bois Diable site, combined with the relatively low height of raised fields (see Sections 6.3.2.2 and 6.3.2.3), could explain why the differences were less clear than at the Bois Diable site. The extent to which the original morphology of raised-field landscapes has been altered by post-abandonment modifications is unclear (McKey et al., 2010; Rostain, 2008a). Raised fields at the K-VIII site could have been higher in the past.

The high concentrations of plant-available K, Mg and Na in ridges at the Piliwa site seem to call into question the idea that leaching of salts from sea water is promoted in raised fields. However, there is evidence that this site is likely flooded by brackish water, and not by fresh water as in the other sites; and that the hydrological conditions at the site today differ from those during pre-Columbian times (see Section SI1.3 in Appendix 2.1).

6.5.3.3 Influences of pre-Columbian fertilization and natural ecosystem engineers on concentrations of Ca, K, Mg and Na

At the Bois Diable and K-VIII sites and in the Organabo savanna, the differences between raised fields and flat areas in the amounts of plant-available cations and pseudo-total Ca, K and Mg were likely caused by differences in clay contents and differing influences of brackish water. Brackish groundwater also apparently influences the amounts of plant-available cations in the Grand Macoua savanna. However, the elevated amount of plant-available Ca in small, scattered round mounds with large intervening flat areas in the Grand Macoua savanna was not accompanied by enhanced values for any other element. Thus, it is unlikely that the enhanced amount of plant-available Ca in these mounds can be attributed to the influence of brackish water. The clay contents of mounds and the flat area were comparable in this savanna (see Section 6.3.2.6).

The data analysed in this study do not allow us to evaluate whether the elevation of Ca in small, scattered mounds with large intervening flat areas in the Grand Macoua savanna could have been caused by addition of material with high Ca content by pre-Columbian farmers. One alternative explanation for this enhancement of Ca in mounds could be the abundance of the termites *Cortaritermes* sp. and *Microcerotermes* sp., which construct carton nests at the soil surface of mounds in this savanna (Renard et al., 2013). Decaëns et al. (2001) found that carton nests of a termite belonging to the genus *Microcerotermes* in a Colombian savanna were especially enriched in plant-available Ca and Mg. The fact that no difference in the amount of plant-available Mg was found could be due to the influence of brackish water in the Grand Macoua savanna on the amount of plant-available Mg. The fact that significantly higher amount of $Ca_{avail.}$ was only found in scattered mounds with large intervening flat areas could be explained by the large dry season foraging areas offered between these type of mounds. Large foraging area per mound could enable termites to occupy a greater proportion of mounds and to maintain larger nests on them. Where mounds are denser, the foraging area per mound is smaller; consequently fewer mounds may be occupied by termite nests.

As found for plant-available P, effects of soil invertebrates on the concentrations of plant-available Ca, K and Mg could have been greater under fire-free agricultural management, as postulated by Iriarte et al. (2012) than under the frequent burning to which most of the sites are subjected today (see Sections 6.5.1 and 6.5.2). Sousa-Souto et al. (2008) showed that, as for plant-available P (see Section 6.5.2), enhancements of the plant-available fractions of Ca, K and Mg in the nests of leaf-cutting ants are also diminished by regular burning of *cerrado* vegetation.

6.5.4 Amounts of pseudo-total Cu, Ba and Zn

Elevated amounts of Cu, Ba and Zn have been generally found to be associated with archaeological sites (Oonk et al., 2009). Elevated amounts of Ba in association with high concentrations of Ca can be indicative of carbonates (De Vos and Tarvainen, 2002) and especially deposition of mollusc shells (Puchelt, 1978). In combination with elevated amounts of P, Zn can be enriched through faeces deposition and Cu through trough urine deposition (Woods, 2003a). However, Ca was not elevated in raised fields at the Bois

Diable and K-VIII sites, and neither a correlation of $\mathrm{Ba}_{\text{pseudo-tot.}}$ with $\mathrm{Ca}_{\text{pseudo-tot.}}$ nor a correlation of $\mathrm{Cu}_{\text{pseudo-tot.}}$ and $\mathrm{P}_{\text{pseudo-tot.}}$ was found. $\mathrm{Zn}_{\text{pseudo-tot.}}$ was correlated with $\mathrm{P}_{\text{pseudo-tot.}}$ only in the upper 10 cm, but at this depth no enrichment of $\mathrm{P}_{\text{pseudo-tot.}}$ was found in raised fields at the Bois Diable and K-VIII sites in comparison to the flat areas. Thus, the pseudo-total amounts of Cu, Ba and Zn did not give evidence for a specific fertilization of raised fields. The correlations of $\mathrm{Cu}_{\text{pseudo-tot.}}$, $\mathrm{Ba}_{\text{pseudo-tot.}}$, and $\mathrm{Zn}_{\text{pseudo-tot.}}$ with TOC, $\mathrm{K}_{\text{pseudo-tot.}}$ and $\mathrm{Mg}_{\text{pseudo-tot.}}$, and the differences between their amounts in raised fields and the associated flat area, indicate that their amounts were related to the contents of SOM and of clay (see Sections 6.3.2.2, 6.3.2.3 and 6.4.2).

6.6 Conclusions

Nutrient concentrations and the amount of TOC in eight types of raised fields at five sites in French Guiana were analysed. Differences in clay content between raised fields and flat areas, which could not completely be avoided during soil sampling, can explain some variation in the element concentrations. Furthermore, amounts of Ca, K, Mg and Na were affected by brackish groundwater at most sites. Variation in their concentrations showed that raised fields in some areas were less affected by brackish groundwater than were flat areas. Elevated amount of TOC not correlating with higher clay content, was found in some raised fields compared to flat areas at depths below 10 cm. This enrichment of TOC could be caused by transfer of organic matter to raised fields by pre-Columbian farmers or by soil invertebrates that inhabit the raised fields. However, at depth below 10 cm, the TOC concentration was not higher than in the upper 10 cm of raised fields and the flat areas. Thus, the elevated amount of TOC in raised fields could also be the result of the construction of raised fields using topsoil material. Variations in the amounts of total N and pseudo-total P were closely correlated with variations in TOC content. Slightly elevated concentration of plant-available P found in some raised fields in comparison to associated flat areas was not correlated with higher clay content. Elevated amount of plant-available Ca found in one type of raised fields compared to an associated flat area was also not correlated with higher clay content nor with the influence of brackish groundwater. However, these differences did not provide clear evidence for fertilization with particularly P- or Ca-rich materials by pre-Columbian farmers, because they could also be attributed to the actions of soil invertebrates that inhabit the raised fields. According to the results of this study, cultivation on raised fields on the nutrient-poor soils (other than cations originating from brackish groundwater) of the coastal savannas of French Guiana is unlikely to have been continuous. Long and frequent fallow periods were probably necessary, so that only a small proportion of the area was under cultivation at a given time. Consequently, population densities are likely to have been lower than the figures put forward in many papers. However, analyses of more specific and persistent parameters will be required to understand the management of nutrients and organic matter in pre-Columbian raised-fields farming system in French Guiana.

Chapter 7

Synopsis and conclusions

7.1 Synopsis

7.1.1 Nutrient sources in terras pretas and steroid analysis

7.1.1.1 Depositions of plant biomass and ash

A number of potential nutrient sources were assumed to be responsible for the high nutrient contents in *terras pretas* and are discussed in Chapter 2. As biochar contains only traces of nutrients, it does not significantly contribute to the nutrient status of *terras pretas* (Glaser, 2007). Arroyo-Kalin et al. (2009) and Woods (2003b) indicated that ash may had been a significant input into *terras pretas*. It is likely that ash was applied on *terras pretas* by human activities. However, it is unlikely that the application of ash was a key process leading to *terra preta* formation as slash-and-burn agriculture does not generate *terra preta* despite the fact that high amounts of ash are applied to such sites. In addition, ash cannot explain the quantities and ratios of nutrients in *terras pretas*. Especially the high enrichment of phosphorus in *terras pretas* must have originated from other material.

It has been hypothesised that plant biomass from rivers and organic matter from floodplains were transported to *terra preta* sites (Denevan, 1996; Glaser, 2007; Lima et al., 2002). Application of high amounts of plant biomass from rivers and organic matter from floodplains could be possible in regions of white water rivers where fertile floodplains (várzea) are found, but does not help explaining the nutrient stocks in numerous *terra preta* sites near blackwater rivers, which are nutrient-poor and support only a floodplain characterized frequently by white sands (Prance, 1979). In addition, like ash, the quantities and ratios of nutrients in *terras pretas* indicate that the deposition of plant biomass was unlikely the key process for *terra preta* genesis.

7.1.1.2 Deposition of animal remains and faeces

Another potential nutrient source is waste, including animal remains such as mammal and fish bones. Mammal and fish bones can even be observed in *terra preta* profiles today. By scanning electron microscopy in combination with energy-dispersive X-ray spectroscopy (SEM/EDS) Lima et al. (2002) and Schaefer et al. (2004) found evidence for high Ca and P derived from bones in some *terras pretas*.

In the study of Chapter 3, the biomarkers for faeces, 5β-stanols, as well as their precursors, Δ^5-sterols, and their 5α-isomers (the main reduction products of Δ^5-sterols in the environment) in five *terras pretas* and reference soils were analysed to investigate the input of faeces into *terras pretas*.

The enrichment of 5α-stanols and Δ^5-sterols in *terras pretas* was not higher than the enrichment of total organic carbon in *terras pretas* compared to the reference soils. Furthermore, the concentrations of 5α-stanols in *terras pretas* and reference soils were correlated with the concentrations of their precursors (Δ^5-sterols). This showed that the distribution of 5α-stanols in *terras pretas* and reference soils could be explained by in situ reduction of Δ^5-sterols. In *terras pretas*, the amount of the faecal biomarker 5β-cholestan-3β-ol was enhanced at 30 – 40 cm soil depth compared to reference soils. The enhancement of 5β-cholestan-3β-ol concentration was more pronounced than the enhancement of cholest-5-en-3β-ol and 5α-cholestan-3β-ol contents. No correlation between 5β-cholestan-3β-ol and cholest-5-en-3β-ol was found. This revealed that the elevated amount of 5β-cholestan-3β-ol in *terras pretas* compared to the reference soils did not originate from in situ cholest-5-en-3β-ol reduction and that 5β-cholestan-3β-ol could be used as a biomarker for faeces in *terras pretas*. Thus, the increased amount of 5β-cholestan-3β-ol in *terras pretas* compared to the reference soils showed that faeces were deposited on *terras pretas* and contributed to their genesis. The steroid data in Chapter 3 are corroborated by compound-specific $\delta^{15}N$ analysis of individual amino acids in a *terra preta* and a reference soil (Chapter 2). These analyses showed that the *terra preta* exhibit more positive $\delta^{15}N$ values of hydrophobic amino acids than the reference soil. Such $\delta^{15}N$ values of amino acids are characteristic for soils fertilized with excrements (Simpson et al., 1997).

7.1.1.3 Method for the combined quantification of faecal stanols, stanones and bile acids in soils and type of faeces deposited on terras pretas

The identification of the type of faeces in *terras pretas* is hindered by limited data of steroid composition of faeces from Amazonian animals. Furthermore, stanols provide only raw information about the origin of the faecal material (Bethel et al., 1994; Bull et al., 1999b, 2002; Evershed and Bethell, 1996; Leeming et al., 1997). However, in contrast to 5β-cholestan-3β-ol being a biomarker for faeces of omnivores (Bethel et al., 1994; Bull et al., 1999b, 2002; Evershed et al., 1997), the stanols which are characteristic for the faeces of herbivore mammals were not enriched in *terras pretas* to the same extent (Chapter 3). Thus, faeces-derived nutrient input into *terras pretas* was most probably derived from omnivores (Chapter 3).

The analysis of bile acids can deliver more detailed information about the source of faeces than the analysis of stanols (Bull et al., 2002; Elhmmali et al., 1997; Evershed and Bethell, 1996; Tyagi et al., 2008). Therefore, a method was developed enabling the quantification of 5β-stanols and their 5α-isomers together with Δ^5-sterols and bile acids based on a single lipid extraction (Chapter 4). 5β-Stanones are assumed to have a potential as biomarkers comparable with 5β-stanols (Grimalt et al., 1990) and their quantification was included in the method.

During the development of this method, amounts of faecal biomarkers analysed with saponification of the total lipid extract and without saponification were compared. This comparison showed that a saponification of the total lipid extract yielded up to 9 times higher amounts of steroids than analyses without saponification. A high variability of the bound proportions was found between and within the samples and no correlations with the different sources of the biomarkers, e.g. faeces vs. other input pathways, were determined. It is possible that the steroids bound to other lipids are stabilized in soils and in terrestrial sediments to a certain degree. Especially with respect to archaeological questions, a more complete extraction could be of interest to include steroids, which are possibly stabilized.

After saponification, sequential liquid–liquid extraction allowed extraction of the biomarkers from the saponification solution and separating the bile acids from the neutral stanols, stanones and Δ^5-steroles without acid-catalysed formation of stanones. Purification of stanols, stanones and Δ^5-sterols was achieved by solid phase extraction using 5% deactivated silica gel. Bile acids were separated from long chain fatty acids by solid phase extraction using activated silica gel after methylation with dry hydrochloric acid in methanol. The relatively unhindered hydroxyl groups of stanols and Δ^5-sterols were silylated with a mixture of hexamethyldisilazane, trimethylchlorosilane and pyridine (3:1:9, v/v/v) avoiding a silylation of the keto groups of the stanones in their enol-form. For bile acids, the stronger silylation mixture N,O-bis(trimethylsilyl)trifluoroacetamide and N-trimethylsilylimidazole (98:2, v/v) was used, which is useful for the derivatisation of hindered hydroxyl groups occurring in bile acids.

The method was evaluated by standard addition to soil samples with a wide range of different physico-chemical properties. The standard contained 5β-stanols, 5 β -stanones and their 5α-isomers together with Δ^5-sterols and bile acids (19 substances). This evaluation showed that the method allows a precise quantification of stanols, stanones, Δ^5-sterols and bile acids with mean recoveries of individual substances ≥85%. The recoveries within each biomarker group did not differ significantly. Thus, the method is suitable for quantification of steroid patterns in samples from soils and terrestrial sediments. Samples from archaeological soils were included in the sample set for method evaluation. Data of these samples showed that the method can be used to determine steroids in an archaeological context.

As part of the method development for the combined analyses of steroid biomarkers for faeces, bile acids in a *terra preta* were analysed (Chapter 2 and Chapter 4). These data showed that the bile acid pattern in *terra preta* was dominated by deoxycholic acid accompanied by relatively high concentrations of lithocholic acid (Chapter 2), typical for faeces of humans (Bull et al., 1999b, 2002). Therefore, all steroid data indicate deposition of human faeces on *terras pretas*.

7.1.1.4 Pre-Columbian land use associated with terras pretas and consequences for the development of sustainable agriculture in the humid tropics

Data reviewed and discussed in Chapter 2 support the hypothesis that *terras pretas* developed from midden areas. Today, midden areas are used as home gardens or home gardens are used as trash areas by indigenous groups (Hecht, 2003; Schmidt and Heckenberger, 2009). Also *terras pretas* are preferentially used for agriculture today (Schmidt and Heckenberger, 2009). Phytolith data has shown that this was the case already in pre-Columbian times (Bozarth et al., 2009). Therefore, it seems likely that agricultural practices in home gardens contributed to the genesis of *terra preta* as well. However, no evidence was found that *terras pretas* developed through fertilization of pre-Columbian fields.

The analyses of steroids in *terras pretas* (Chapter 3) suggest an on-side deposition of human excrements. Consequently, faecal material should be considered as input path of nutrients into *terras pretas* when phosphorus stocks and the amounts of other nutrients in *terras pretas* are used to calculate population densities in pre-Columbian Amazonia. Faecal material is not only a nutrient-rich organic material, the application of faeces to agricultural soils also causes shifts in their microbial community structure (Enwall et al., 2007; Maeder et al., 2002; Marschner et al., 2003; Peacock et al., 2001; Sun et al., 2004). The microbial community structure of *terras pretas* differs from the microbial communities in surrounding soils (Kim et al., 2007; O’Neill et al., 2009; Ruivo et al., 2009). The deposition of faeces could have favored this microbial community structure in *terras pretas* which is assumed to have changed soil nutrient and carbon dynamics in ways that promoted the sustainability (Lehmann, 2009). Therefore, faeces could be an important fertilizer for a sustainable agriculture in the humid tropics which imitates properties of *terra preta*.

7.1.2 Nutrients in raised fields and nutrient management in pre-Columbian raised-field agriculture in Amazonia

7.1.2.1 Soil organic matter, nutrients and nutrient sources

In Chapter 5 it is shown that fertilization with material originating from external sources was probably a component of pre-Columbian raised-field agriculture in Meso- and South America in areas outside of Amazonia. Some soil micro-morphological features in raised fields in Ecuador indicate the application of animal manure (Wilson et al., 2002). In Mesoamerica, compost, probably including faeces, was used to fertilize raised fields in the Valley of Mexico (Armillas, 1971). In the study of Chapter 6, nutrient concentrations and amounts of total organic carbon in raised fields, belonging to all types of mounds and ridges previously described in French Guiana (Rostain, 2008a, 2010; McKey et al., 2010), were analysed and their properties compared to those of soils in flat savanna areas in the surrounding area. Differences in clay contents between raised fields and flat areas, which could not completely be avoided during soil sampling, can explain some variation in the element concentrations. Furthermore, amounts of Ca, K, Mg and Na were affected by brackish groundwater at most sites. Variation in their concentrations showed that raised fields in some areas were less affected by brackish groundwater than were flat areas.

An elevated amount of total organic carbon was found in raised fields at some sites compared to flat areas in the surrounding area. Only at one site, the elevated amount of total organic carbon was correlated with higher clay content of raised fields compared to the associated flat area. In Chapter 5 it is assumed that slashed biomass of fallow vegetation and biomass and litter from the basins between raised fields may have been applied onto raised fields in Amazonia. It is known that crop residues are incorporated into contemporary analogues of raised fields outside of Amazonia (Crews and Gliessman, 1991; Denevan and Turner, 1974; Domingo and Hagerman, 1982; Kirch, 1978; Kleinhenz, 1997). Phytoliths of vegetative parts of maize, found in raised fields in French Guiana (Iriarte et al., 2010), could indicate mulching with crop residues. Thus, the enhanced amounts of total organic carbon in some raised fields compared to associated flat areas could have originated from addition of slashed plant biomass and litter onto raised fields by pre-Columbian farmers. The total organic carbon concentration in raised fields was higher than in the associated flat areas only at depths below 10 cm (at the sites, where the differences in total organic carbon were not correlated with differences in clay content). At these depths, total organic carbon concentration was lower than in the upper 10 cm of mounds and the associated flat areas. Such an elevation of total organic carbon could have been simply caused by the addition of topsoil material onto raised fields during their construction. Additionally, transfer of sediments in combination with vegetation from the basins between raised fields to the cropping area could have caused this elevation of total organic carbon. Such transfer is an inevitable consequence of maintaining raised fields, and is common in analogous systems outside of Amazonia (Crews and Gliessman, 1991; Denevan, 2001; Vasey et al., 1984). However, it is hard to distinguish the addition of slashed plant material and litter from the transfer of basin sediments in combination with plant material to raised fields during their maintenance and construction.

In addition to the direct movement of material with high organic matter content by pre-Columbian farmers, soil invertebrates could also have contributed to higher total organic carbon concentrations in raised fields compared to the associated flat areas. McKey et al. (2010) described the transport of organic matter by ants and termites to their nests in raised fields. An increase of organic matter in ant nests has frequently been reported (Frouz and Jilková, 2008). Analyses of soil invertebrates in small raised fields showed that termites of the subfamily Nasutitermitinae are the most abundant termites in these raised fields (McKey et al., 2010; Renard et al., 2013). Higher total organic carbon contents in termite mounds than in surrounding soils have been found in nests of various mound-building termites, including Nasutitermitinae, e.g. in savannas in Colombia (Decaëns et al., 2001), in secondary forests in central Amazonia (Ackerman et al., 2007) and in pastures in Minas Gerais, Brazil (Sarcinelli et al., 2009). Additionally, earthworms belonging to the genus Martiodrilus were found in small raised fields (Renard et al., 2013). Studies in the Eastern Plains of Colombia showed that organic matter is stabilized in the casts of Martiodrilus earthworms (Guggenberger et al., 1996; Mariani et al., 2007). Thus, natural ecosystem engineers could also have contributed to enhanced total organic carbon contents in raised fields.

Variations in the amounts of total N and pseudo-total P were closely correlated with variation in total organic carbon content. Slightly elevated concentration of plant-available P found in some raised fields in comparison to associated flat areas were not correlated with higher clay content. Elevated amount of plant-available Ca found in one type of raised fields compared to an associated flat area were also not correlated with higher clay content nor with the influence of brackish groundwater.

While the small enhancement in the concentration of plant-available P could conceivably be indicative of fertilization of raised fields with materials with high P content, this enhancement could also be due to the activities of natural ecosystem engineers inhabiting the raised fields. The activity of soil macrofauna generally enhances the availability of P (Seastedt, 1995). The availability of P is usually higher in earthworm casts and ant nests than in soils without these structures (Chapuis-Lardy et al., 2011; Frouz and Jilková, 2008). Phosphorus availability is often enhanced in termite mounds, but data are sometimes contradictory and materials used by termites in feeding and nest-building have a strong influence on P availability (Chapuis-Lardy et al. 2011; Decaëns et al., 2001; López-Hernández et al., 2006; Rückamp et al., 2010; Sarcinelli et al., 2009). A number of studies have reported that enhanced availability of P in biogenic structures is correlated with neutral pH values (Frouz and Jilková, 2008; Sarcinelli et al., 2009). Differences in pH value between raised fields and the associated flat areas were small (Chapter 6). However, earthworms of the family Glossocolecidae, *Pontoscolex corethrurus* Müller and *Martiodrilus* sp., were the most frequently found species in raised fields (Renard et al., 2013). In a rainforest Oxisol in Brazil, higher P availability in the casts of Glossoscolecidae was not associated with higher pH values (Kuczak et al., 2006). Specifically, enhanced P availability in the casts of the earthworm *Pontoscolex corethrurus* Müller is assumed not to be associated with changes of pH in the cast (Lopez-Hernandez et al., 1993).

The data analysed in this study did not show whether the elevation of Ca in one type of raised fields could have been caused by addition of material with high Ca contents by pre-Columbian farmers. One alternative explanation for this enhancement of Ca in mounds could be the abundance of the termites *Cortaritermes* sp. and *Microcerotermes* sp., which construct carton nests at the soil surface of mounds in this savanna (Renard et al., 2013). Decaëns et al. (2001) found that carton nests of a termite belonging to the genus *Microcerotermes* in a Colombian savanna were especially enriched in plant-available Ca and Mg. The fact that no differences in the amounts of plant-available Mg were found could be due to the influence of brackish water on the amounts of plant-available Mg. The fact that significantly higher amounts of plant-available Ca were only found in one type of raised fields could be explained by the specific morphology of this raised-field complex (see discussion in Chapter 6).

7.1.2.2 Methods for the extraction of elements

The discussion of the plant-available and pseudo-total nutrient concentrations in raised fields in Chapter 6 shows that slight differences in the plant-available element fractions that are not accompanied by a total or pseudo-total enhancement are hard to interpret in the context of anthropogenic deposition of nutrient-rich materials. Slight differences in the plant-available fraction caused by human soil modifications compared to soils of reference areas may be overwhelmed by much higher natural background concentrations obtained by pseudo-total and total extractions (Holliday and Gartner, 2007; Middleton, 2004; Middleton and Price, 1996; Wilson et al., 2006). However, the analyses of nutrients in raised fields and flat areas (Chapter 6) showed that small differences in easily extractable element concentrations can theoretically only deliver clear indications of anthropogenic deposition of nutrient-rich materials if all effects that influence their availability would be quantitatively analysed in the investigated soils.

7.1.2.3 Nutrient management in pre-Columbian raised-field agriculture in Amazonia

Analogous to the properties assigned to areas that are suspected to be vestiges of pre-Columbian agricultural fields on the non-flooded *terra firme*, (*terras mulatas*; see Section 1.2), the biogeochemical legacy of pre-Columbian raised-field agriculture in French Guiana did not provide clear evidence that pre-Columbian farmers fertilized the raised fields with particularly nutrient-rich materials from an external source (Chapter 6). Nutrient and total organic carbon analyses in raised fields in French Guiana showed that cultivation on raised fields on the nutrient-poor soils (other than cations originating from brackish groundwater) of the coastal savannas of French Guiana is unlikely to have been continuous. Long and frequent fallow periods were probably necessary, so that only a small proportion of the area was under cultivation at a given time. Consequently, population densities are likely to have been lower than the figures put forward in many papers (Iriarte et al., 2010; McKey et al., 2010; Rostain, 2008a, 2010).

As discussed in Chapter 5, similar to shifting cultivation on the *terra firme*, during fallow periods, build-up of plant biomass could be the source of most of the nutrients that permit growth of crops on pre-Columbian raised fields. In seasonally flooded savannas, biomass build-up is much lower than in upland forests. However, in Chapter 5 it is discussed that the wetland component may have played an important role for organic matter and nutrient management in raised-field agriculture. Wild plants adapted to waterlogging probably could grow less disturbed and more abundantly than during cultivation on raised fields. Water logging generally enhances the availability of P through reduction of iron oxides and further processes. Especially in very wet basins, litter accumulates from algae and aquatic macrophytes with relatively low C:N and C:P ratios. Consequently, it is concluded in Chapter 5 that plant biomass and litter from basins could have been an important source of nutrients in raised fields.

Today, the savannas are fire-prone environments. Iriarte et al. (2012) postulated that pre-Columbian raised-field agriculture in French Guiana was largely fire-free. Probably a reduction of the frequency and intensity of fires during fire periods could have accelerated biomass build-up during fallow periods (Chapter 5). Additionally, Lavelle (1978) in Lavelle and Spain 2001) showed that anecic earthworms incorporate less litter in regularly burned savannas in the Ivory Coast than in less burned savannas. Furthermore, Sousa-Souto et al. (2008) showed that enhancement of plant-available P, Ca, K and Mg in the nests of leaf-cutting ants in Brazilian savannas (cerrado) is diminished by regular burning. These data indicate that nutrient concentration on raised fields by soil invertebrates could have been more pronounced when burning was limited (see discussion in Chapter 6).

7.2 Conclusions

Available data show that the high fertility of *terras pretas* was caused, in addition to the incorporation of charred organic matter (biochar), by the deposition of several materials with high nutrient content. It is likely that ash and plant biomass contributed to the high nutrient stocks. However, the deposition of ash or plant biomass was unlikely the key process for *terra preta* genesis. Waste including animal remains such as mammal and fish bones are likely to contribute to the nutrient stocks. The analyses of biomarkers for faeces, 5β-stanols, as well as their precursors, Δ^5-sterols, and their 5α-isomers in *terras pretas* and reference soils showed that elevated amount of 5β-cholestan-3β-ol in *terras pretas* compared to the reference soils did not originate from in situ cholest-5-en-3β-ol reduction and that 5β-cholestan-3β-ol could be used as biomarker for faeces of omnivores in *terras pretas*. Thus, the increased amount of 5β-cholestan-3β-ol in *terras pretas* compared to the reference soils showed that faeces of omnivores were deposited on *terras pretas* and contributed their genesis. A method was developed enabling a precise quantification of faecal biomarkers from different groups (stanols, stanones, Δ^5-sterols and bile acids) in soils with mean recoveries of individual substances ≥85%. Data from a *terra preta* showed that the bile acid pattern in the *terras pretas* is typical for faeces of humans Therefore, all steroid data indicate deposition of human faeces on *terras pretas*. However, review of literature data indicate that nutrient-rich materials were not intentionally applied on *terras pretas* to improve soil fertility at large scales, and all data fit to a genesis of *terras pretas* from midden eventually combined with home garden agriculture.

Enhanced amounts of total organic carbon and nutrients in some pre-Columbian raised fields in French Guiana compared to flat areas could also be attributed to the actions of soil invertebrates that inhabit the raised fields and to the construction of the raised fields by pre-Columbian farmers using topsoil material.

No evidence was found showing clearly that pre-Columbian farmers intentionally fertilized agricultural fields with nutrient-rich material from external sources. Cultivation on raised fields on the nutrient-poor soils (other than cations originating from brackish groundwater) of the coastal savannas of French Guiana is unlikely to have been continuous. Long and frequent fallow periods were probably necessary, so that only a small proportion of the area was under cultivation at a given time. Consequently, population densities are likely to have been lower than the figures put forward in many papers. During fallow periods, reduction of fire frequency and build up of plant biomass in the basins between raised fields could had been of great importance for the management of nutrients in pre-Columbian raised-field agriculture. However, faeces should be considered as nutrient source in estimations using nutrient amounts in *terras pretas* to calculate population densities in pre-Columbian Amazonia and they could be an important fertilizer for a sustainable agriculture in the humid tropics which imitates properties of *terras pretas*.

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Appendices

Appendix 1 (Chapter 4)

Equation for the calculation of relative standard deviation (RSD)

The following equation was used to calculate the RSDs from the data of the standard addition experiment (section 4.3.12.4).

$$\mathrm{RSD} = \sqrt{\frac{1}{\mathrm{N}-1} \cdot \sum_{j=1}^{n} \sum_{i=1}^{k} \frac{(\mathrm{m}_{\text{subsample, i, j}} - \mathrm{m}_{\text{s tan dard, i, j}} + \overline{\mathrm{m}_{\text{s tan dard, j}}} - \overline{\mathrm{m}_{\text{soil, j}}})^2}{\overline{\mathrm{m}_{\text{soil, j}}}^2}}$$

N := number of measurements

n := number of soils j (one sample of each soil)

k := number of subsamples i of the soil j

$\mathrm{m}_{\text{sample, i, j}}$:= amount in the TLE of the subsample i,j

$\mathrm{m}_{\text{standard, i, j}}$:= amount of standard added into the TLE of the subsample i,j

$\overline{\mathrm{m}_{\text{standard, j}}}$:= mean amount of standard added to the TLE of the subsamples of the soil j

$\overline{\mathrm{m}_{\text{soil, j}}}$:= mean amount in the TLE of the subsamples of the soil j (the different amounts of added standards to the TLE were not subtracted for the calculation of this mean)

Appendix 2 (Chapter 6)

Appendix 2.1
SI1 Genesis, archaeological background and distribution of sampled raised fields

SI1.1 Bois Diable site

Large mounds at the Bois Diable site consisted of topsoil and mixed topsoil and subsoil material. The topsoil horizon was 50 cm thick. Below the topsoil horizon followed a horizon of mixed topsoil and subsoil material (7 cm). Below the mixed horizon a buried topsoil horizon was found. Organic matter of the uppermost part of the buried topsoil horizon dated to 750 ± 40 ^{14}C y B.P. (670-700 Cal y B.P., McKey et al., 2010). This date is broadly contemporaneous with the dates of Barbakoeba residential sites, one of which, was found close to the raised fields (McKey et al., 2010). Phytholiths of maize (*Zea mays* L.) found in these mounds indicate that they were used as raised fields (Iriarte et al., 2010; McKey et al., 2010).

SI1.2. K-VIII site

Different types of large raised fields found in this site were arranged over very slightly sloping terrain in a highly organized fashion, as described by McKey et al. (2010) and Rostain (2010). In the best-drained upper part of the complex, elongate ridges were oriented parallel to elevation contours, an orientation favouring water retention. Down slope, elongate ridges were oriented along the slope, suggesting this configuration was designed to facilitate drainage. Round raised fields were situated in the flat very low-lying area below the slope.

The raised fields consisted of topsoil and mixed topsoil and subsoil material. The topsoil horizon was 10 – 20 cm thick. Below this, mixed topsoil and subsoil material was found. A buried topsoil horizon was found at a depth of ca. 45 – 55 cm.

The organic matter of the uppermost sector of the buried topsoil horizon dated to 1,010 ± 40 ^{14}C y B.P. (920-950 Cal y B.P., McKey et al., 2010) which is broadly contemporaneous with the dates of Barbakoeba residential sites (McKey et al., 2010). Phytholiths of maize (*Zea mays* L.) were found in the raised fields, showing their past agricultural use (Iriarte et al., 2010; McKey et al., 2010).

SI1.3. Piliwa site

In large ridges at the Piliwa site, virtually no topsoil material was found below 10 cm depth down to 40 cm. Furthermore, this part of the ridges was dominated by greyish colours with less reddish mottling compared to soils in other areas. A dark horizon, possibly a buried topsoil horizon, was found at 40 – 50 cm depth in these ridges. Phytoliths of maize (*Zea mays* L.) and of cultivated squash (*Cucurbita* sp.) found in these ridges show that they were used for agriculture (Iriarte et al., 2010; McKey et al., 2010).

Rostain (2008) stated that the raised fields in this site are flooded completely during the rainy seasons and concluded that the hydrological conditions at this site today may differ from those during pre-Columbian times. The Piliwa site is located very close to the estuary of the Mana river in an area where large shoreline changes occurred during the last two centuries (Plaziat and Augustinus, 2004). A marine transgression during the last decades (Institut Géographique National, 2004; Plaziat and Augustinus, 2004) could have led to sea-water intrusion into the basin where the ridges are located. The site is thus likely flooded by sea or brackish water and not by fresh water as are the other sites.

SI1.4. Organabo savanna

Topsoil material was only found in the upper 5 – 10 cm of the medium-sized mounds in the Organabo savanna. Below a gradual transition to subsoil horizons at 35 cm depth was observed. Formation of these mounds has not been analysed. However, phytoliths of maize were found in this mounds (J. Iriarte, unpubl. data), showing that the area was used for agriculture in the past.

SI1.5. Grand Macoua Savanna

In this site, entire small mounds consisted of topsoil material, with topsoil continuing down to ca. 10 – 20 cm below the base of each mound. Phytolith and carbon-isotope data, in combination with the analysis of the recent vegetation, indicate that the mounds in the Grand Macoua savanna were the result of building-up processes and not the result of preferential erosion of the soil between the mounds (Renard et al., 2012). Their arrangement in a square-grid in some areas and in striking geometric patterns in some areas give evidence that the mounds were built by humans (McKey et al., 2010; Renard et al., 2012). Phytholiths of maize (*Zea mays* L.) were found in these mounds, indicating that they were used as raised fields (Iriarte et al., 2010; McKey et al., 2010).

SI1.6. Location of the sampled raised fields

The locations of the sampled raised fields are shown in figure SI1.1. The coordinates are provided in Section 6.3.2

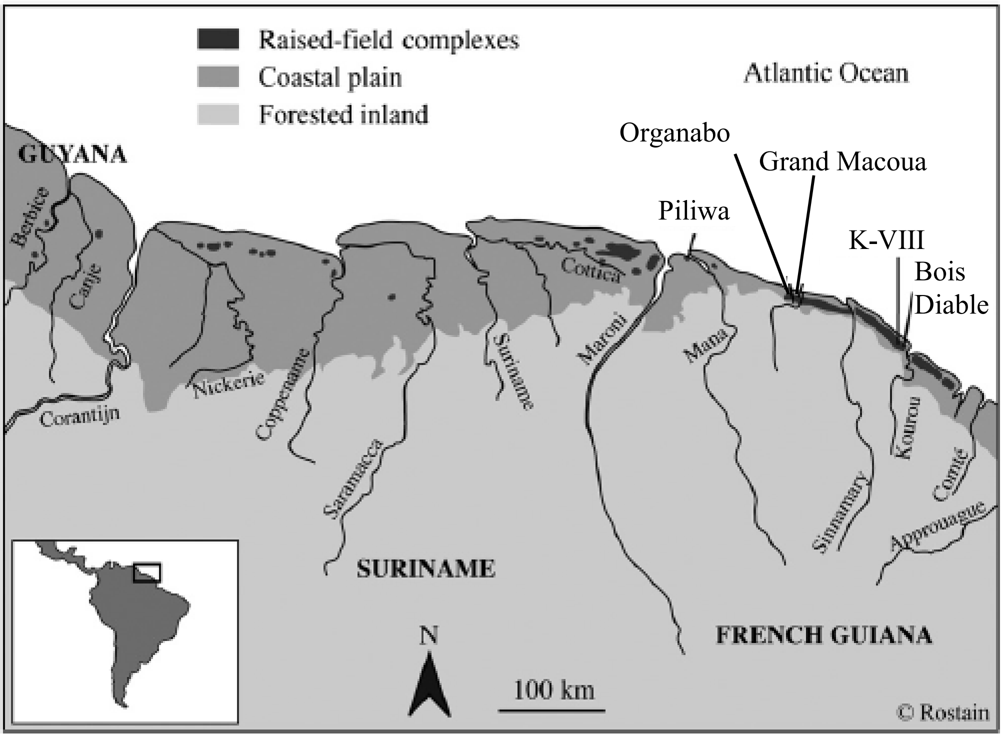

Figure SI1.1 Map of the Guianas showing the distribution of raised fields and the locations of the sampled raised fields (modified from McKey et al., 2010).

SI1.7 References

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Appendix 2.2

SI2 Figures

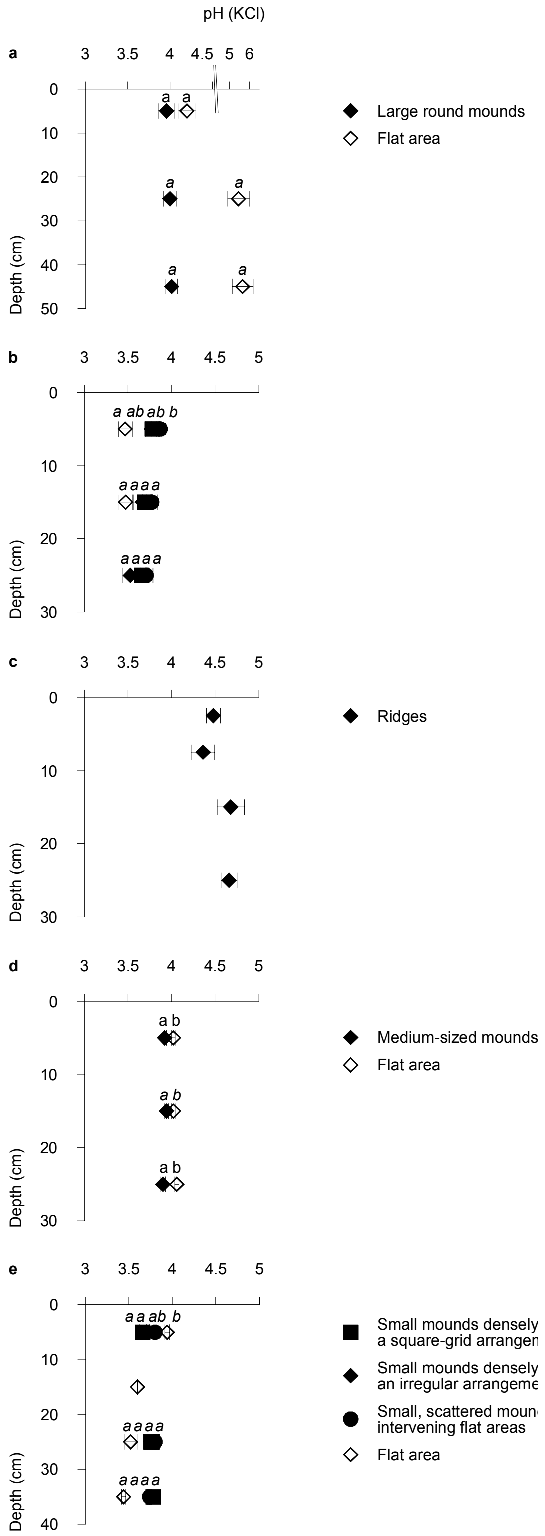

Figure SI2.1 Mean pH measured in KCl in raised fields and flat areas. (a) Bois Diable site, (b) K-VIII site, (c) Piliwa site, (d) Organabo savanna, (e) Grand Macoua savanna [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, $n = 5$].

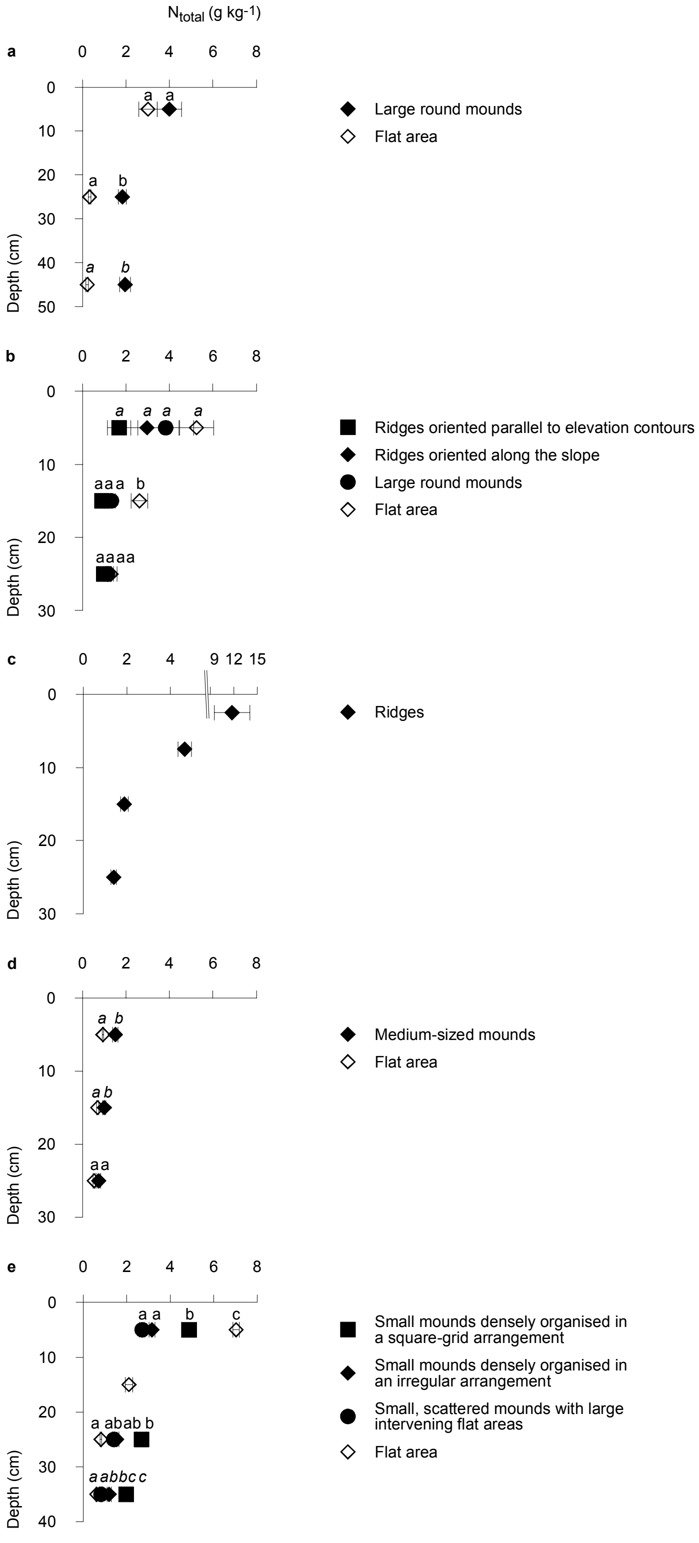

Figure SI.2 Mean total amount of nitrogen ($N_{tot.}$) in raised fields and flat areas. (a) Bois Diable site, (b) K-VIII site, (c) Piliwa site, (d) Organabo savanna, (e) Grand Macoua savanna [different letters indicate significant differences ($P < 0.05$), letters in italics denote results of non-parametric tests, error bars show standard errors, n = 5].

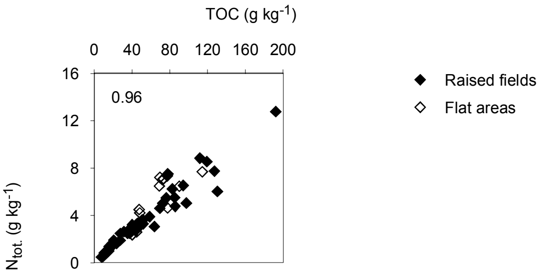

Figure SI2.3 Correlation between the amounts of total nitrogen ($N_{tot.}$) and of total organic carbon (TOC) at 0 - 10 cm depth, in raised fields and flat areas at all sites. Spearman's rank correlation coefficient for raised fields and flat areas taken together, R = 0.96 (P < 0.05).

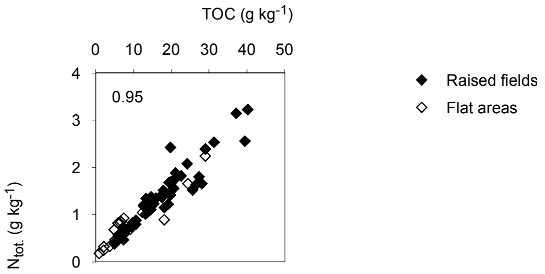

Figure SI2.4 Correlation between the amounts of total nitrogen ($N_{tot.}$) and of total organic carbon (TOC) at 20 - 30 cm depth, in raised fields and flat areas at all sites. Spearman's rank correlation coefficient for raised fields and flat areas taken together, R = 0.95 (P < 0.05).

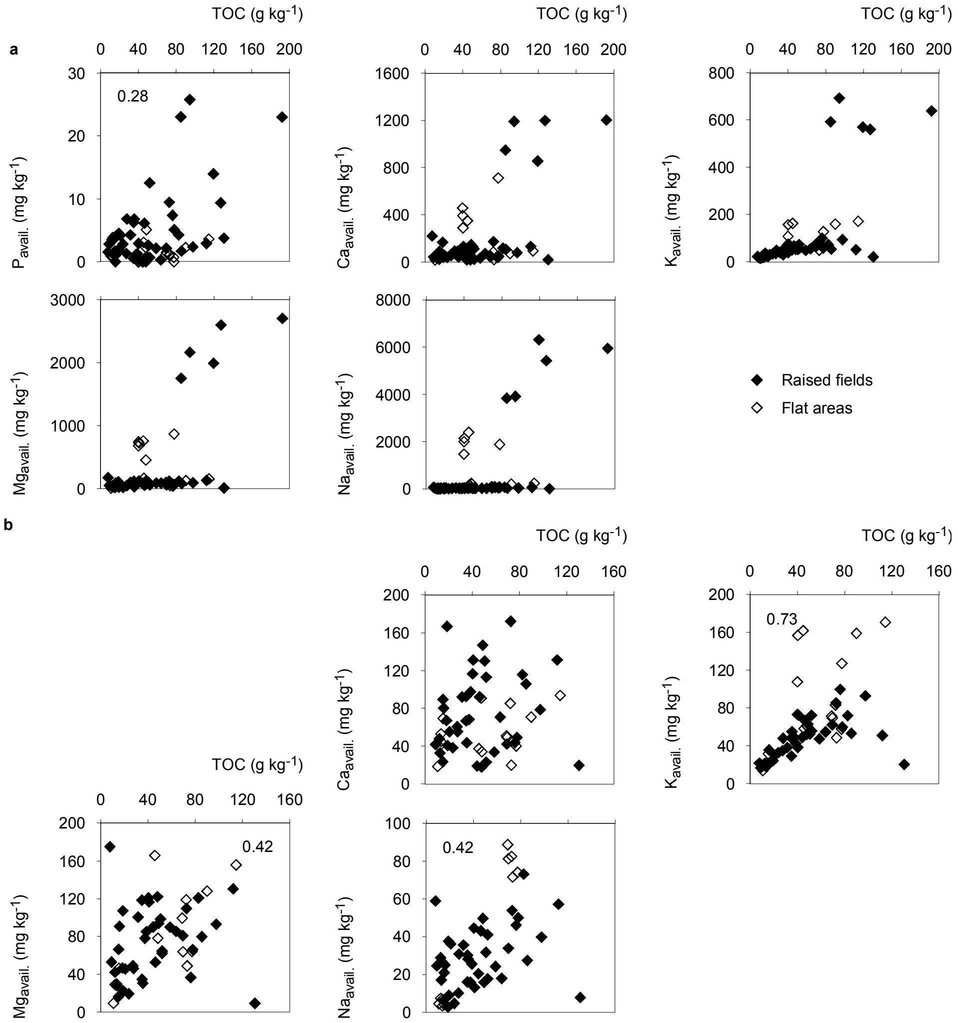

Figure SI2.5 Correlations between plant-available amounts of phosphorus (P$_{avail.}$), calcium (Ca$_{avail.}$), potassium (K$_{avail.}$), magnesium (Mg$_{avail.}$) and sodium (Na$_{avail.}$) and amounts of total organic carbon (TOC) at 0 - 10 cm depth, in raised fields and flat areas at all sites, (a) all data and (b) including only samples with amounts of Ca$_{avail.}$ < 200 mg kg^{-1}, K$_{avail.}$ < 200 mg kg^{-1}, Mg$_{avail.}$< 400 mg kg^{-1}, and Na$_{avail.}$ < 150 mg kg^{-1} (to avoid misinterpretations caused by a cluster with outstandingly high values, section 2.4). Numbers show Spearman's rank correlation coefficients (P < 0.05) for raised fields and flat areas taken together.

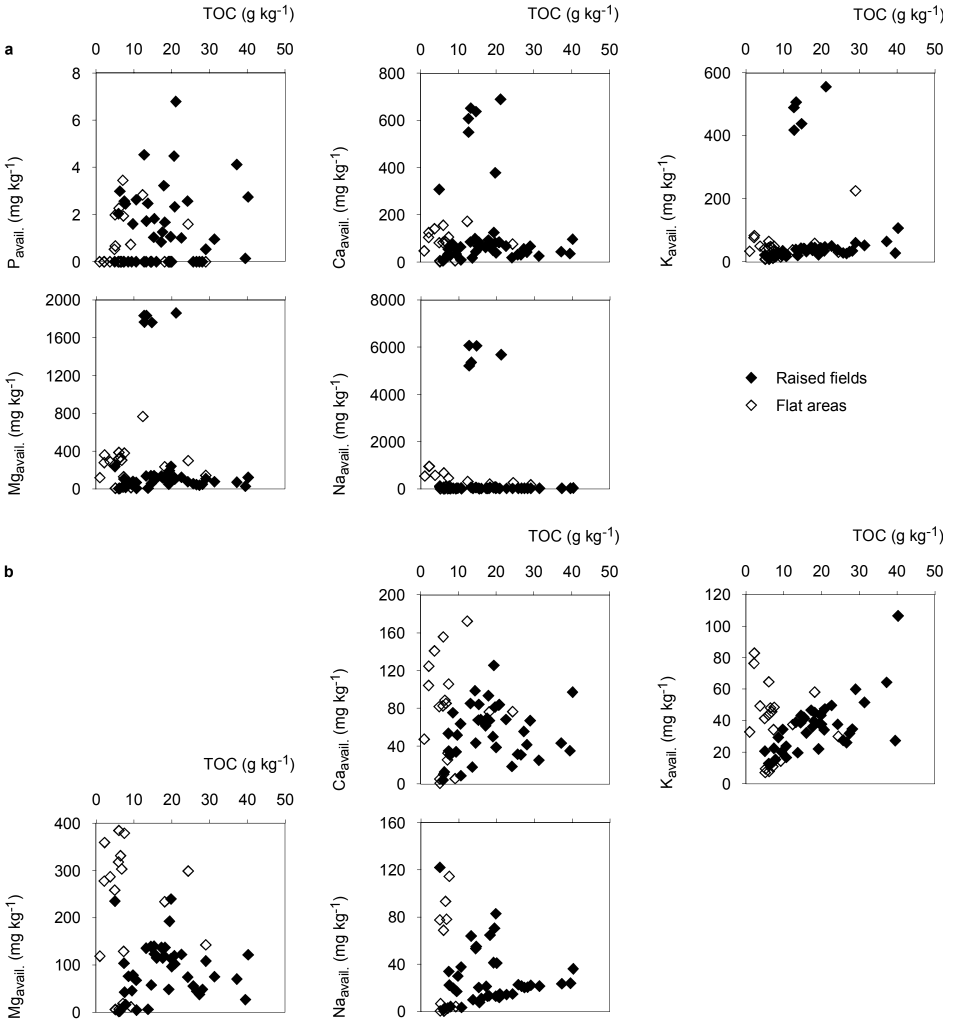

Figure SI2.6 Correlations between plant-available amounts of phosphorus ($P_{avail.}$), calcium ($Ca_{avail.}$), potassium ($K_{avail.}$), magnesium ($Mg_{avail.}$) and sodium ($Na_{avail.}$) and amounts of total organic carbon (TOC) at 20 - 30 cm depth, in raised fields and flat areas at all sites, (a) all data and (b) including only samples with amounts of $Ca_{avail.}$ < 200 mg kg^{-1}, $K_{avail.}$ < 200 mg kg^{-1}, $Mg_{avail.}$< 400 mg kg^{-1}, and $Na_{avail.}$ < 150 mg kg^{-1} (to avoid misinterpretations caused by a cluster with outstandingly high values, section 2.4).

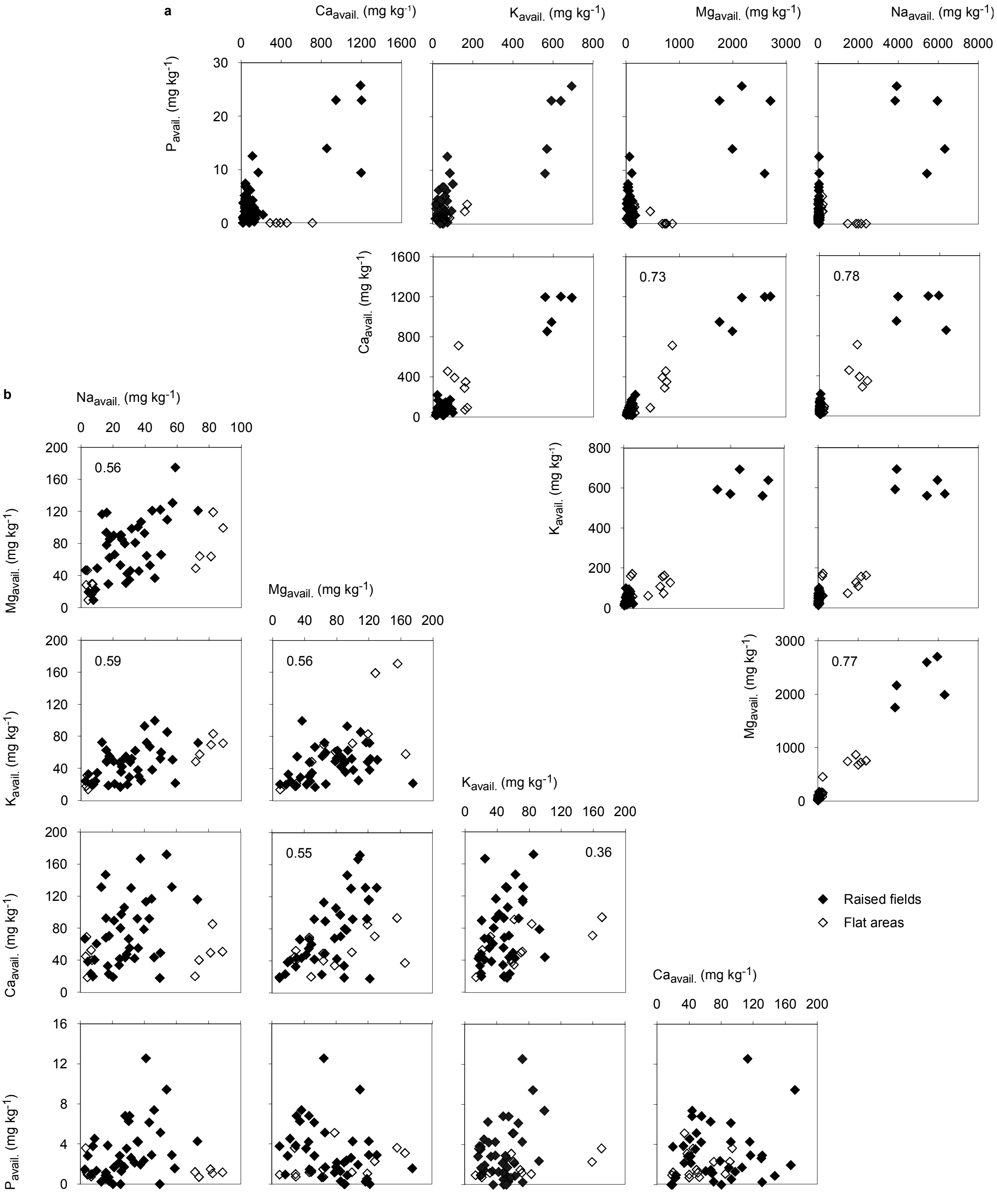

Figure SI2.7 Correlations between plant-available amounts of phosphorus ($P_{avail.}$), calcium ($Ca_{avail.}$), potassium ($K_{avail.}$), magnesium ($Mg_{avail.}$) and sodium ($Na_{avail.}$) at 0 - 10 cm depth, in raised fields and flat areas at all sites, (a) all data and (b) including only samples with amounts of $Ca_{avail.} < 200$ mg kg^{-1}, $K_{avail.} < 200$ mg kg^{-1}, $Mg_{avail.} < 400$ mg kg^{-1}, and $Na_{avail.} < 150$ mg kg^{-1} (to avoid misinterpretations caused by a cluster with outstandingly high values, section 2.4). Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

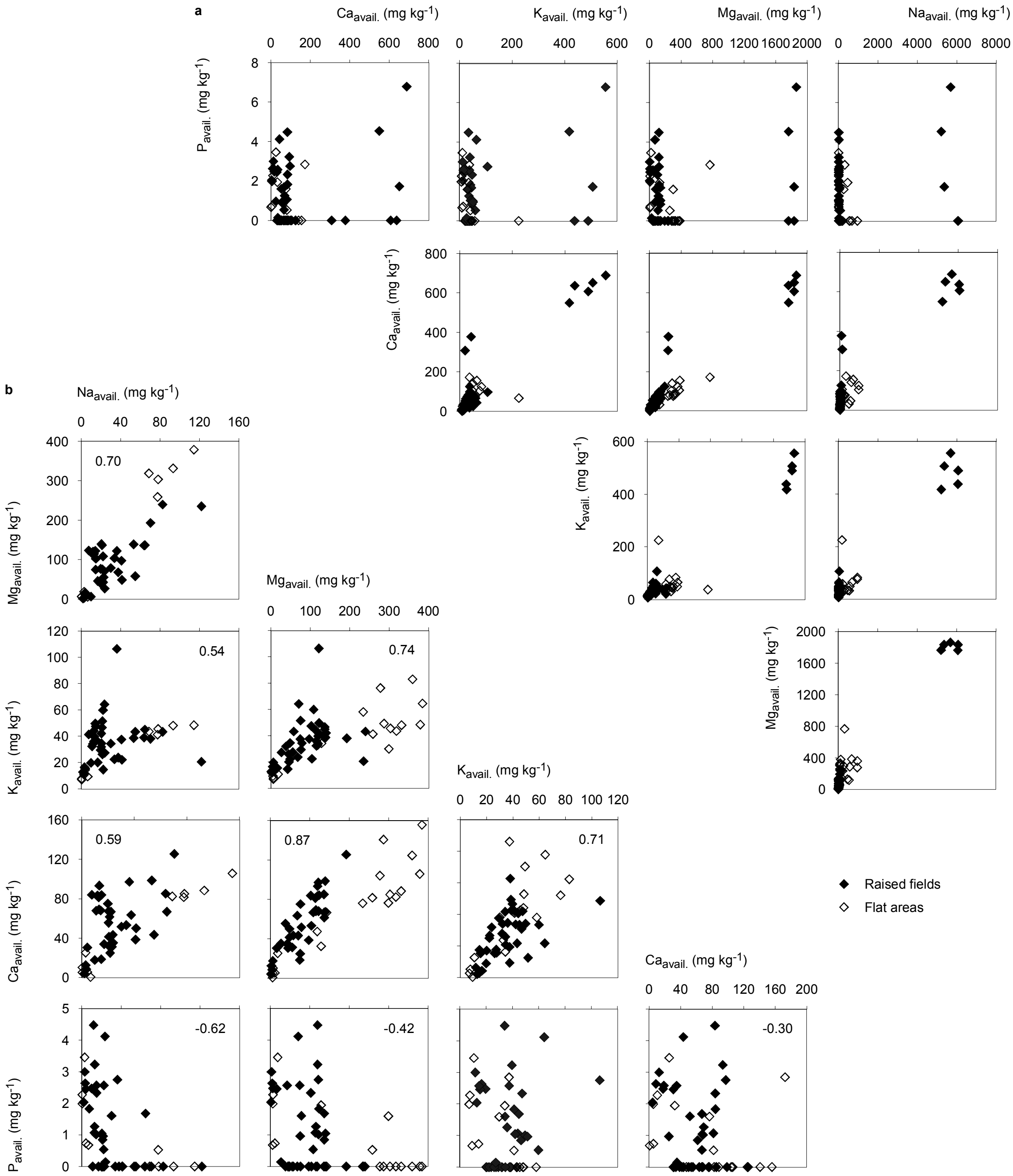

Figure SI2.8 Correlations between plant-available amounts of phosphorus ($P_{avail.}$), calcium ($Ca_{avail.}$), potassium ($K_{avail.}$), magnesium ($Mg_{avail.}$) and sodium ($Na_{avail.}$) at 20 - 30 cm depth, in raised fields and flat areas at all sites, (a) all data and (b) including only samples with amounts of $Ca_{avail.}$ < 200 mg kg^{-1}, $K_{avail.}$ < 200 mg kg^{-1}, $Mg_{avail.}$ < 400 mg kg^{-1}, and $Na_{avail.}$ < 150 mg kg^{-1} (to avoid misinterpretations caused by a cluster with outstandingly high values, section 2.4). Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

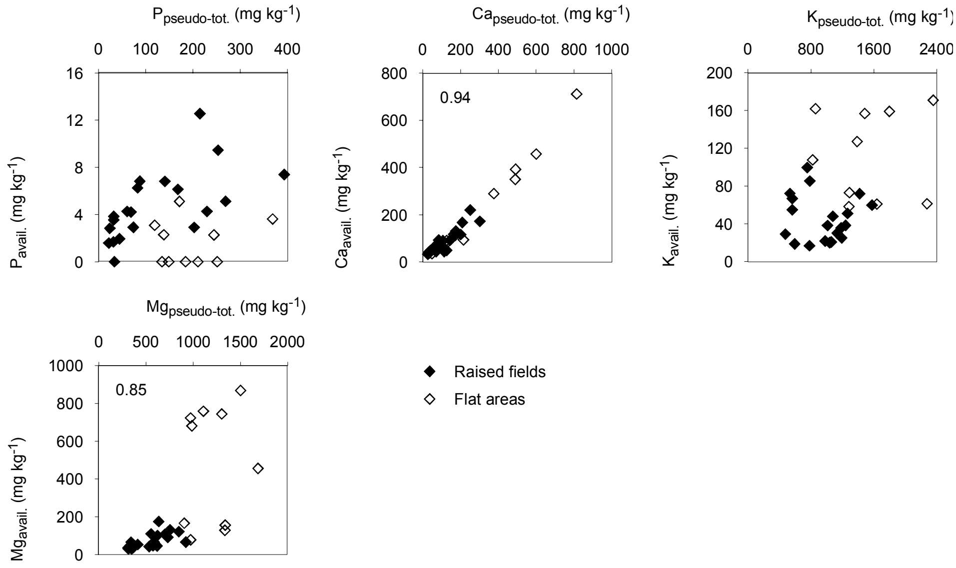

Figure SI2.9 Correlations between pseudo-total amounts and plant-available amounts of phosphorus ($P_{pseudo\text{-}tot.}$ vs. $P_{avail.}$), calcium ($Ca_{pseudo\text{-}tot.}$ vs. $Ca_{avail.}$), potassium ($K_{pseudo\text{-}tot.}$ vs. $K_{avail.}$) and magnesium ($Mg_{pseudo\text{-}tot.}$ vs. $Mg_{avail.}$) at 0 - 10 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

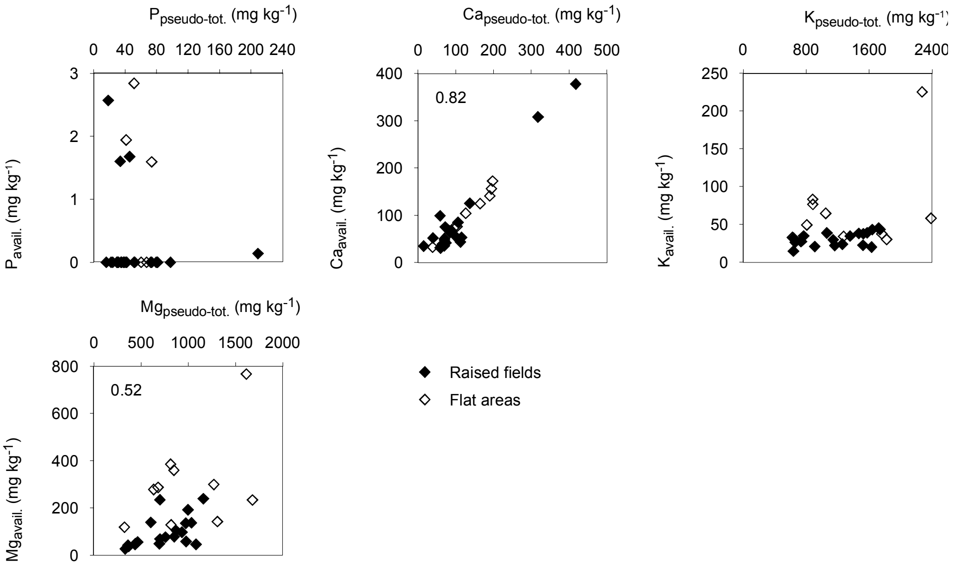

Figure SI2.10 Correlations between pseudo-total amounts and plant-available amounts of phosphorus ($P_{pseudo\text{-}tot.}$ vs. $P_{avail.}$), calcium ($Ca_{pseudo\text{-}tot.}$ vs. $Ca_{avail.}$), potassium ($K_{pseudo\text{-}tot.}$ vs. $K_{avail.}$) and magnesium ($Mg_{pseudo\text{-}tot.}$ vs. $Mg_{avail.}$) at 20 - 30 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

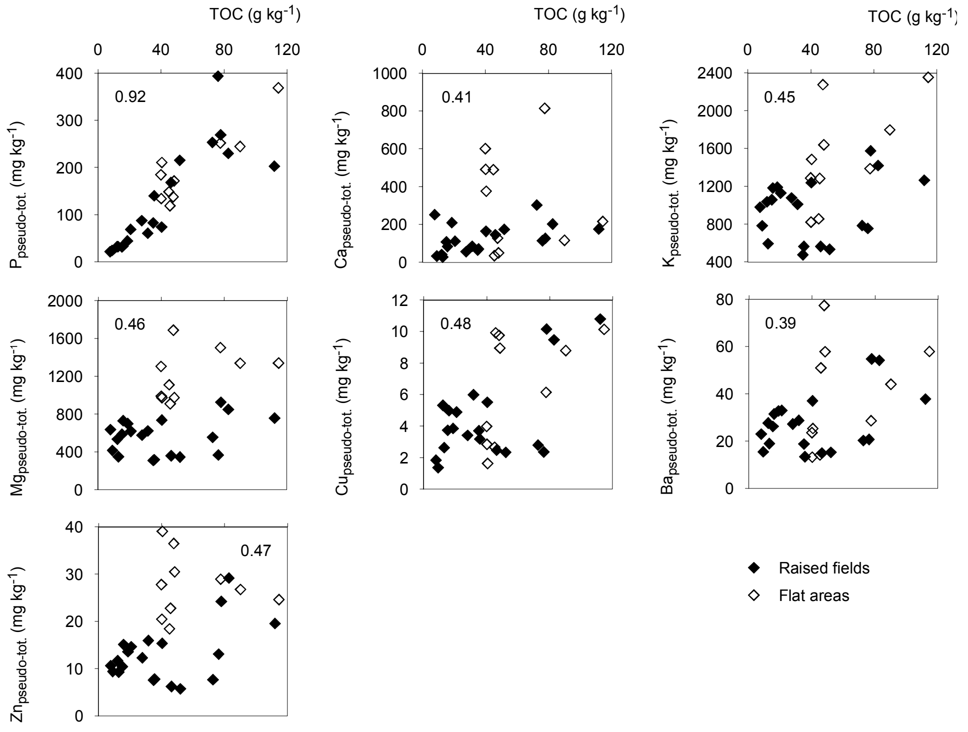

Figure SI2.11 Correlations between pseudo-total amounts of phosphorus ($P_{pseudo\text{-}tot.}$), calcium ($Ca_{pseudo\text{-}tot.}$), potassium ($K_{pseudo\text{-}tot.}$), magnesium ($Mg_{pseudo\text{-}tot.}$), copper ($Cu_{pseudo\text{-}tot.}$), barium ($Ba_{pseudo\text{-}tot.}$) and zinc ($Zn_{pseudo\text{-}tot.}$) and amounts of total organic carbon (TOC) at 0 - 10 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

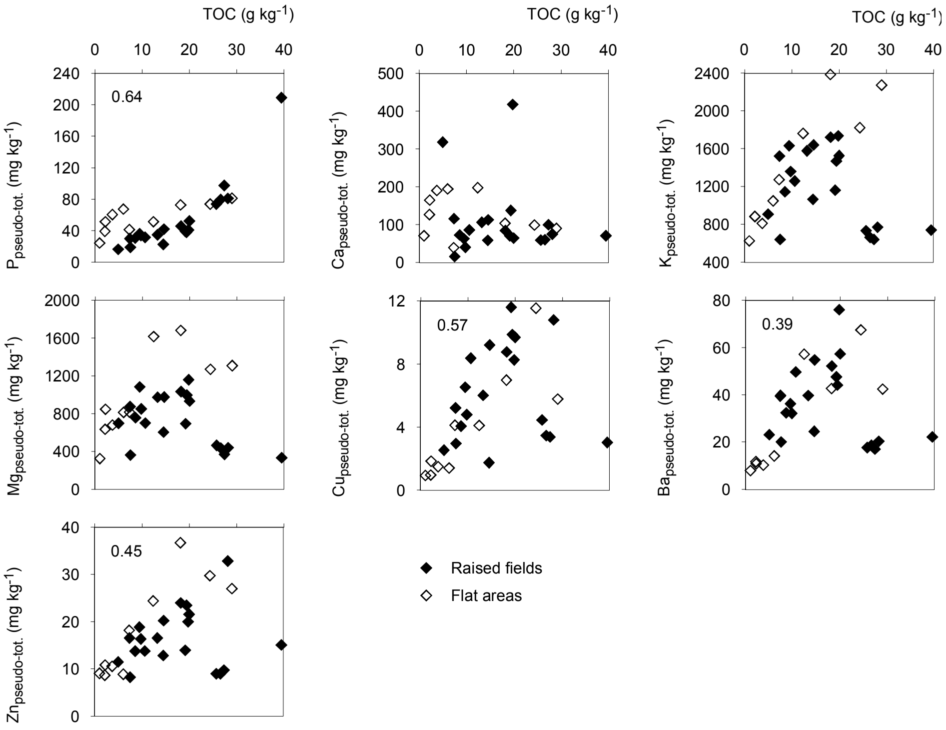

Figure SI2.12 Correlations between pseudo-total amounts of phosphorus ($P_{pseudo\text{-}tot.}$), calcium ($Ca_{pseudo\text{-}tot.}$), potassium ($K_{pseudo\text{-}tot.}$), magnesium ($Mg_{pseudo\text{-}tot.}$), copper ($Cu_{pseudo\text{-}tot.}$), barium ($Ba_{pseudo\text{-}tot.}$) and zinc ($Zn_{pseudo\text{-}tot.}$) and amounts of total organic carbon (TOC) at 20 - 30 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

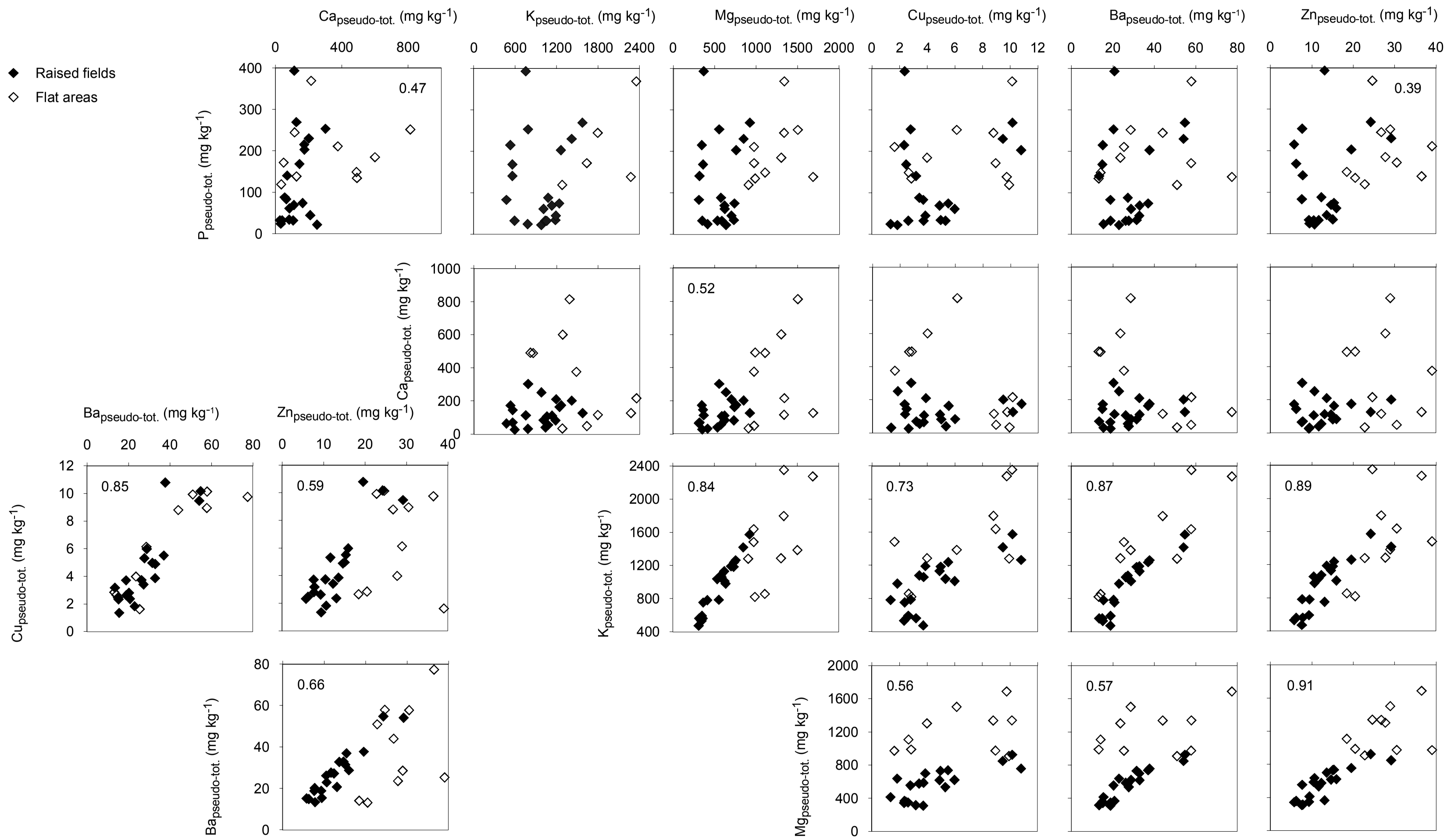

Figure SI2.13 Correlations between pseudo-total amounts of phosphorus (P$_{pseudo-tot.}$), calcium (Ca$_{pseudo-tot.}$), potassium (K$_{pseudo-tot.}$), magnesium (Mg$_{pseudo-tot.}$), copper (Cu$_{pseudo-tot.}$), barium (Ba$_{pseudo-tot.}$) and zinc (Zn$_{pseudo-tot.}$) at 0 - 10 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

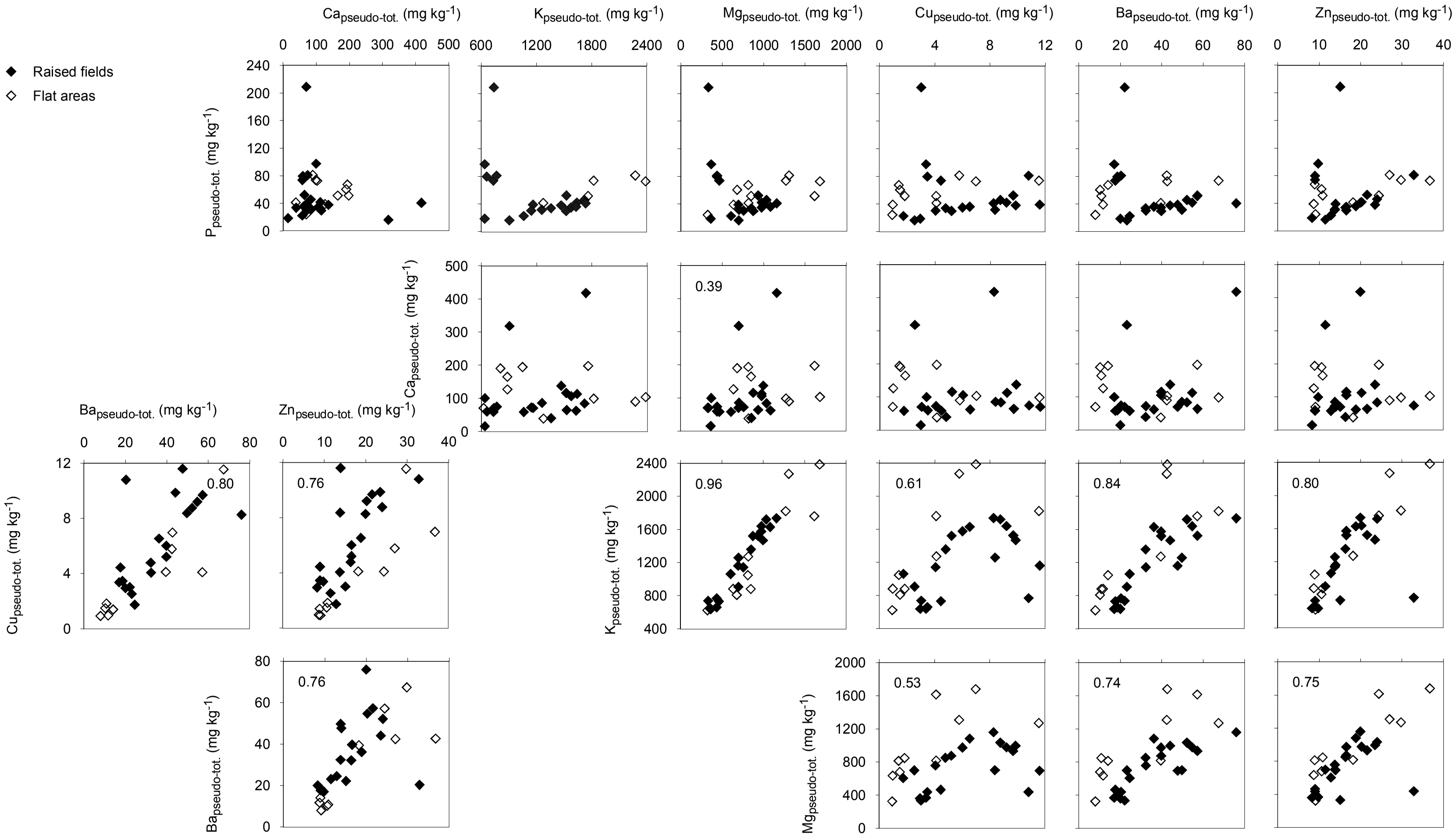

Figure SI2.14 Correlations between pseudo-total amounts of phosphorus ($P_{pseudo\text{-}tot.}$), calcium ($Ca_{pseudo\text{-}tot.}$), potassium ($K_{pseudo\text{-}tot.}$), magnesium ($Mg_{pseudo\text{-}tot.}$), copper ($Cu_{pseudo\text{-}tot.}$), barium ($Ba_{pseudo\text{-}tot.}$) and zinc ($Zn_{pseudo\text{-}tot.}$) at 20 - 30 cm depth, in raised fields and flat areas at the Bois Diable site and the K-VIII site. Numbers show Spearman's rank correlation coefficients ($P < 0.05$) for raised fields and flat areas taken together.

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Eidesstattliche Erklärung / *Declaration under Oath*

Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst, keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.
I declare under penalty of perjury that this thesis is my own work entirely and has been written without any help from other people. I used only the sources mentioned and included all the citations correctly both in word or content.

Datum / Date

Unterschrift des Antragstellers / *Signature of the applicant*