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# Origin of nutrients in Amazonian Dark Earths as assessed by molecular markers

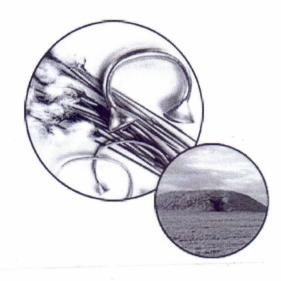
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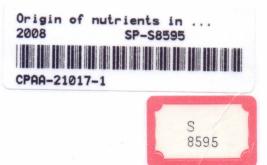
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### Abstract

Geochemically, Amazonian Dark Earths are characterised by a high fertility caused by stable soil organic matter and high nutrient levels. The nutrient stocks and nutrient forms have been intensively investigated in the last decades. Only a few studies about the origin of nutrients have been carried out. Up to now, bones have been identified as one source of nutrients in Amazonian Dark Earths but other sources, e.g. plant biomass and faecal material, are still a matter of speculation. We will discuss possible nutrient sources and associated landuse practices, and will present the first data from analyses of stanols and bile acids. These biomarkers are used to investigate the input of faecal material to soils. Our data show that excrements contribute to the fertility of Amazonian Dark Earths. Analytical procedures and the applicability of this method, which as far as we know has not been used before in the humid tropics, will be discussed.

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# Origin of nutrients in Amazonian Dark Earths as assessed by molecular markers

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Geochemically, Amazonian Dark Earths are characterised by a high fertility caused by stable soil organic matter and high nutrient levels. The nutrient stocks and nutrient forms were intensively investigated in the last decades. However, only few studies about the origin of nutrients were carried out. Up to now, bones have been identified as one source of nutrients in Amazonian Dark Earths but other sources like e.g. plant biomass and feacal material are still a matter of speculation. We will discuss possible nutrient sources and associated landuse practices and will present first data from analyses of stanols, and bile acids. These biomarkers are used to investigate the input of faecal material to soils. Our data show that excrements contribute to the fertility of Amazonian Dark Earths. Analytical procedures and the applicability of this method, which was as far as we know, not used before in the humid tropics, will be discussed.

#### Introduction

The main geochemical feature which distinguishes Amazonian Dark Earths (ADE) from surrounding Acrisols and Ferralsols is a high fertility caused by stable soil organic matter and high nutrient levels (Glaser et al. 2001b; Lehmann et al. 2003; Glaser 2007). Whereas nutrient stocks and nutrients forms were intensively investigated in the last decades only few studies about the origin of nutrients were carried out. With respect to potential nutrient sources, only nitrogen can be "produced" biologically *in situ* via nitrogen fixation. Other elements such as phosphorus, potassium, calcium, zinc and magnesium must be incorporated from the surroundings for nutrient accumulation (Glaser 2007). *In situ* weathering as a source of these elements can be excluded in Amazonia, at least on the heavily weathered Ferralsols and Acrisols, since these do not contain high concentrations of these elements (Glaser 2007).

Waste including mammal and fish bones and products of combustion and charring likely contribute to the nutrient stocks in ADE. Lima et al. (2002) found evidence of high calcium and phosphorus linked to bones in Amazonian Dark Earth using scanning electron microscopy and energy-dispersive X-ray 165 spectroscopy. Glaser et al. (2001a) showed that Amazonian Dark Earths contain high amounts of black carbon which is linked to charcoal. There is no analytical proof that great amounts of plant biomass was applied on ADE. Often it is hypothesised that plant biomass from rivers and crop residues from floodplains were transported to ADE sites. This could be possible in regions of white water rivers where fertile floodplains (*várzea*) are found but does not help to explain the nutrient stocks in ADE near

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black water rivers which are nutrient poor and support only a floodplain which is characterised frequently by white sands.

Our work focuses on faecal material as one possible nutrient source. Manuring with excrements was wide spread in pre-historic cultures and is described for South America in the Andes (Denevan 1995). Biomarker analyses offers the opportunity to detect ancient manuring in soils. I detail, coprostanol and its epimer epi-coprostanol in relation to cholestanol can be used to detect faecal input to soils (Bull et al. 1999; Bull et al. 2002). Coprostanol mainly forms by microbial metabolism of cholesterol in the gut of omnivore mammals and can be further transformed to epi-coprostanol in soil. In contrast, by microbial processes in soils the majority of cholesterol is transformed into cholestanol and only a minor amount of cholesterol is reduced in soils to coprostanol. Due to the problem that coprostanol and epicoprostanol are not completely specific, their amounts are related with the amounts of cholestanol to get a molecular faecal indicator. Similar to coprostanol, 5 $\beta$ -campestanol und 5 $\beta$ stigmastanol can be used to measure manuring with the faeces of herbivore mammals (Bull et al. 1999; Bull et al. 2002). Furthermore it is possible to distinguish between different groups of mammals (e.g. herbivores, pigs and human) by analysing bile acid patterns (Evershed and Bethell 1996; Bull et al. 1999; Bull et al. 2002).

Due to the stability of stanols and bile acids they can be used as biomarkers for ancient manuring (Simpson and al. 1998; Simpson et al. 1999; Bull et al. 2001). We used these compounds to test the hypothesis that faecal material was applied on ADE and present first data from ongoing analyses.

#### **Material and Methods**

Five profile pairs (ADE – Ferralsol) were sampled near Manaus and Santarem (Brazil).  $\Delta^5$ sterols and stanols were analysed according to the method described by Isobe et al. (2002) and bile acids were analysed according to the method used by Elhmmali et al. (1997), Simpson et al. (1999), Elhmmali et al. (2000) and Bull et al. (2003) with small modifications for both.

#### Results

In ADE soils the amounts of coprostanol and epi-coprostanol were elevated compared to the reference soils. But also cholesterol and cholestanol were more abundant in ADE (data not shown).

We evaluated the applicability of theses biomarkers to investigate ancient manuring in ADE by the calculation of correlation coefficients. Cholestanol is assumed to be formed from cholesterol in soils (Bull et al. 1999; Bull et al. 2002). This fits with high correlation coefficients between this substances in ADE (Figure 1). In contrast coprostanol correlated considerable less with cholesterol (Figure 1). Therefore we assume different sources for coprostanol and cholestanol in ADE and interpret that coprostanol was introduced by faeces application whereas cholestanol originated from directly applied tissue.

This interpretation is underlined by the ratio of coprostanol and epi-coprostanol contents to the sum of cholestanol, coprostanol and epi-coprostanol contents. In the upper 10 cm this ratio did not distinguish ADE from reference soils, but was significantly higher in ADE in 30 - 40 cm soil depths (Figure 2) showing application of omnivore faeces. The lack of difference in the shallowest 10 cm of the topsoil could be caused by recent land use, which effects a dilution of the ancient stanol contents.

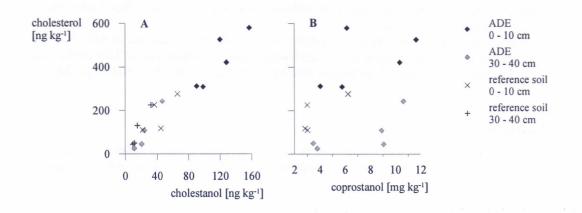


Figure 1: Correlations of cholesterol vs. colestanol (A; ADE 0 - 10 cm:  $R_p = 0.89$ , ADE 30 - 40 cm:  $R_p = 0.97$ ; reference soils 0 - 10 cm:  $R_p = 0.73$ ; reference soils 30 - 40 cm:  $R_p = 0.73$ ) and cholesterol vs. coprostanol (B; ADE 0 - 10 cm:  $R_p = 0.50$ , ADE 30 - 40 cm:  $R_p = 0.73$ , reference soils 0 - 10 cm:  $R_p = 0.78$ ) in ADE and reference soils (N = 5).

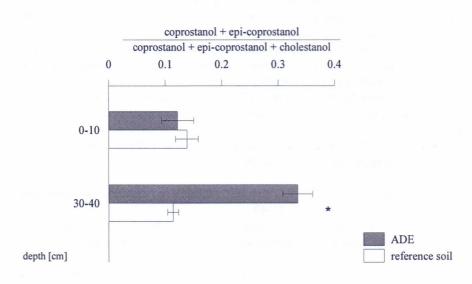


Figure 2: Sum of the amounts of coprostanol and epi-coprostanol divided by the amounts of cholestanol, coprostanol and epi-coprostanol in ADE and Ferralsols. A high value indicates application of faecal material (\* indicates significant differences (P < 0.05) between ADE and refrence soils; error bars show standard errors; N = 5)

Characteristic biomarkers for faeces of herbivore mammals were only found sporadically and only in traces in our soils.

First test measurements of bile acids in ADE yielded a poor recovery but in ADE bile acid patterns are dominated by deoxycholic acid with minor concentrations of lithocholic acid. This is a characteristic pattern for human faecal material (Bull et al. 1999; Bull et al. 2002).

## Conclusions

Our first results showed that stanols can be used to investigate faecal material in ADE and that omnivore faecal material was applied on ADE. This faecal material appears to be of human origin.

#### Acknowledgements

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