Capítulo 16

POPULATION AND BIODIVERSITY IN AMAZONIAN DARK EARTHS SOILS

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Introduction

Many aspects of the origin of Amazon Dark Earths (ADE) are still unclear. The knowledge about the past of the land use practices of Amazon inhabitant and induced soil processes that may have caused the sustainable fertility of the (ADE).

The analysis of amazons anthropogenic soils indicate that the alterations caused by human actions, such as incorporation of organic residues and the effects of fire in the superficial horizon influenced some of the chemical (Carbon, Phosphorus) and physical (aggregation) characteristics (Kern 1996, Glaser 1999, Ruivo & Cunha 2003, Ruivo et al. 2004a).

The ADE overlying deep weathered kaolinitic soils of Amazon represents one of the most interesting features of the Amazon Basin, with broad implications to human ecology. It's chemical composition and forms of phosphate and potassium, micropedological attributes, and their ecological implications (Ruivo et al. 2004a, Schaefer et al. 2004).

Soil is the most important habitat for microorganisms. The biology of ADE soils is important. Little is currently known about the abundance, activity and diversity of organisms extant in ADE. So, we have much to learn (Thies & Suzuki 2004). Is very important the study of local knowledge of soil and land management in an ecological perspective in the Amazon Region. The aspects relationships at soil microbiology are important for the soil

fertility management. The analyze of the past and present can be *favorable* researches and recommendations are given on how ethnopedological studies can contribute to enhance sustainable land use and management in Soil Amazon (Lehmann et al. 2004, Winkler-Prins & Barrera-Bassols 2004, Thies & Suzuki 2004).

Soil consists of a variety of surfaces that influence nutrients availability and affect interactions between different microorganisms. Pores of various sizes are available for exploitation and colonization. The organic matter occurs as freshly added plant, animal, and insect remains, which gradually transformed into stabilized nutrient-rich humus material. These varied components form heterogeneous aggregates of various sizes called *peds*, which contain a complex network of pores. Bacterial and Fungi use different functional strategies to take advantage of these complex physical matrix. Most soil bacteria are located on the surface of soil particles and require water and nutrients that must be located in their immediate vicinity. Bacteria are found most frequently on surfaces within smaller soil pores. Here they are probably less liable to be eaten by protozoa, unlike bacteria that are located on the exposed outer surface of a sand grain or organic matter particle.

The filamentous fungi, in contrast, tend to be located on the outside of the aggregates. These organisms, with their filamentous growth will form bridge between separated regions where moisture is available. The filamentous fungi can move nutrients and water over greater distances in soil. Protozoa, soil insects, nematodes, and other soil animals contribute to the formation and maintenance of soils (Prescott et al. 1999).

In Amazon there are soils called Archeological Black Earth (ABE), Amazon Dark Earths (ADE) or Indian Black Earth (IBE), which have higher fertility than those of its surroundings (Kern 1996). This superior degree of fertility of ABE is, possibly, related to the great diversity and amount of the species, which constitute its micro flora.

This study is aimed to evaluate the microbial community density and biomass microbial of various ADE zones through colony counting of bacteria and fungi as well as by their genus identification. It is little contribution the study of the biodiversity in ADE Soils.

Dark Earths Soils Localization

The ADE sites of the investigation were localized in:

1. The Scientific Station Ferreira Penna is located in the Caxiuanã National Forest, in Melgaço, 350 km west from Belém City – Pará State. The *terra firme* forest, one of the most representative's natural ecosystems in the Amazon Region, covers the region. The soils are characterized as yellow Latosoil (Oxisol in U.S. Department of Agriculture Soil Taxonomy), but have a laterite layer approximately 2 - 4 m below the surface. They vary from well to very well drained and the texture varies from sandy to clay (Ruivo & Cunha 2003).

2. The Santarém City in the area of the Tapajós River, Pará State, under silviculture, site Embrapa-Cpatu, localized in Belterra. The soil is clayed texture.

3. In area de Amazon River. Pará State the areas in study are located in the village of Tabatinga, Juruti-Pará. In places of Sao Felipe, Maravilha (Reinaldo Coelho), Romão and Sao Francisco. In the place of Sao Felipe had an orchard of oranges and sleeves, then after more or less into years 50 one changed into pasture for cattle and culture of gram patchouli, remaining until the present. Its ground has a sand texture. The site Maravilha (Reinaldo) was chosen for study of the ground for presenting a long time of culture, where it becomes culture rotation the 37 years, without any product aiming at the improvement of the ground, this ground has clayed texture. The locality Romão presents one ground in zone of transition between TPA and adjacent ground has as sandy texture. The area of Sao Francisco was opened in adjacent ground to TPA ground, with clayed and sandy texture.

4. Area of the Amazon Rivers in Manaus City, Amazonas State under terra firme forest. The samples collected in colon they had in its majority a texture is sand composition.

Characterization of the Soils

The sample ABE soils were collected in surface in also soils: Caxiuanã (0-10 cm and 10-20 cm of depth), Santarém/Belterra (0-20 cm of depth), Juruti/Tabatinga (0-5 cm; 5-10 cm and 10-20 cm of depth), Manaus (0-5 cm; 5-10 cm and 10-20 cm of depth). These soils were predominantly sandy texture. In also soils the similar mineralogy (kaolin and quartz) in the soil matrix and high fertility. The complete soils characterization and description were in Ruivo & Cunha 2003, Kern 1996 and Ruivo et al. 2004.

For comparison of the population microbial include in the work results of the Yellow Latosoil from Roraima State (area Yanomami/Homoxi) under sandy and clay soils and Site of the Project LBA- Esecaflor in Caxiuanã.

Soils Analyses

A. Micro morphology Analysis

Soil samples from Caxiuanã and Santarém were observed for their micro mineralogy and micro morphology with scanning electron microscopy.

B. Biological Analysis

Microbial population

Soil samples from Caxiuanã, Santarém and Manaus were observed for microbial population. The bacteria and fungi counting was carried out by the Pour-Plate method using the Plate Count Agar (PCA) and Acidified Potato Dextrose Agar (PDA) media, incubated at 35°C and 25°C for 24 hours and 5 days respectively. The number of bacteria and fungi was determined by the colony-forming unit technique with assistance of a Colonies Counter (CP-602). The predominant colonies were isolated and their morphological characters observed in optical microscopy.

The bacteria isolation was made by the use of the Brain Heart Infusion Agar media for the individual colony growth. The genus identification was achieved through microscopic examination of colony and cell morphology, by Gram staining and also by biochemical tests such as: VM-VP, Starch, Gelatin, NH_3, H_2S, Indole, Sugar Fermentation, Oxidase, Catalase and Nitrate.

For fungi isolation and identification was used the Sabouraud at 2% Agar media in wet chamber cultivation and after the colonies' growth were made microscope observations using the lacto phenol cotton blue in order to stain the cellular morphology, mycelia and reproductive structures.

Microbial biomass

Soil samples from of the different places of the Juruti/Tabatinga were analyzed using the method of the fumigation extraction (Vance & Brookes 1987) since 25 g of soil samples were conditioned in desiccators and then submitted at fumigation with chloroform (alcohol free) during 24 hours. After fumigation, chloroform was removed by successive aspirations. Following, the soil samples were agitating during 30 minutes in 0,5 M K_2SO_4 and then filtrated in Whatman 42 paper. For no fumigated samples were made extraction with 0,5 M K_2SO_4 and posterior filtrated. The carbon of the microbial biomass (CBM) of fumigated and not fumigated extracts had been made by titration (dichromatemetry), according to De-Polli & War 1999, from the same extract was made the determination of nitrogen of the microbial biomass (NBM), in Kjeldahl for distillation the vapor (De-Polli & War 1999), the factor used for determination of the NBM was 0,26 (Brookes et al. 1985). The CO2 evolution (activity microbial) produced in the breath of the microorganisms was made following the methodology proposal for Grengorich et al. 1990. O CBM and NBM were calculated by formulas:

$$CBM = \frac{Cfumigated - Cnofumigated}{K_c} \qquad Kc = 0,26$$

$$NBM = \frac{Nfumigated - Nnofumigated}{K_c} \qquad Kc = 0,54$$

Results

Population

The counting results of ADE sites samples from Caxiuanã (Portel County-PA), Santarém County (PA) and outskirts of Manaus (AM) show remarkable predominance of bacteria population over fungi's (Tables 1-2). This bacteria number superiority is likely due to factors such as greater metabolic versatility and fast growth.

The differences between ADE microbial population density and other kinds of soils are depicted in Table 1. In compared the microbiota of the Yellow Latosoil in the Dark Earths showed

higher diversity, including a distinct more number of the fungal and bacteria genus, very occurrence of the actinomycetes, more occurrence of the organic substances and micelles distribution. This result is shows in ADE soils from Caxiuanã, Santarém and Manaus that compared at soils of the Roraima and Pará (Caxiaunã) (Table 1). These organisms, important decomposers of organic matter, in the Dark Earths have more occurrences and more production of the organic substances and micelles.

The identification tests showed the presence of Gram-negative bacteria of the *Achromobacter*, *Flavobacterium*, *Nitrobacter*, *Nitrosomonas*, *Pseudomonas*, *Escherichia*, *Enterobacter* and *Celovibrio* genera; and Gram-positive bacteria of the *Arthrobacter*, *Bacillus*, *Micrococcus*, *Streptomyces* and *Sarcina* genera. Among these genera we can find cellulolytic, humic acid producers, lignin decomposers, starch decomposers and nitrogen producers.

As to fungi were identified the genera: *Rhizopus*, *Rhizomucor*, *Trichoderma*, *Cladosporium*, *Penicillium*, *Mucor*, *Aspergillus*, *Fusarium* and *Chaetomium*.

Microbial Biomass

We results indicate that soils ABE have difference value of the carbon and nitrogen biomass microbial (Tables 3-4). Comparisons between farms in Juruti/Tabatinga (Table 3) show sites Farm differences also in depth. In the soils from Manaus (Table 4), shows also differences between depths. The results of the Manaus are more for C-BMS, but CO_2 flux (microbial activity) is higher in Juruti. In this place have more nutrients for decomposition of the material culture. The value of the CO_2 concentration in places of Juruti, indicative of the activity microbial, present differences between farms.

Table 1

Microbial Population in substrate of gravel (SG), Yellow Latosol (YL), Red Yellow Latosol (RYL) and Archaelogical Black Earth (ADE)

Substrate	Bacteria	Funguses Moulds	Actinomycetes Yeasts	
		CFU/g of soil		
SG with no vegetation	32×10^4	17×10^4	80×10^3	2×10^4
SG with vegetation	89×10	4×10^4	..	2×10^3
YL.R. quei.	2×10^n	7×10^3	..	5×10^3
YL cap	121×10^4	$20{,}9 \times 10^4$	40×10^4	..
RYL FOR	12×10^4	$4{,}7 \times 10^4$	7×10^4	..
ADE Sant.	32×10^4	38×10^4	80×10^4	2×10^3
ADE Cax. (0-10cm)	54×10^4 to 213×10^4	6×10^4 to 42×10^4	63×10^4 to 108×10^4	2×10^3 to 3×10^3
ADE Cax. (0-20cm)	120×10^4 to 258×10^4	12×10^4 to 18×10^4	185×10^4 to 25×10^5	

The samples SC and YL are proceeding from Roraima State. The ABE samples are proceeding from Pará State.

Table 2

Bacteria and fungi population countings from ADE soils in the surroundings of Manaus City - Amazonas State - Brazil

ADE Soil Code Sample	Bacteria CFU/g of soil	Fungi
733	17×10^{6}	10×10^{4}
734	7×10^{6}	11×10^{4}
735	3×10^{6}	1×10^{4}
736	7×10^{6}	53×10^{4}
737	1×10^{6}	16×10^{4}
738	9×10^{6}	23×10^{4}
739	3×10^{6}	10×10^{4}
740	2×10^{6}	5×10^{4}
741	6×10^{6}	2×10^{4}
742	13×10^{6}	20×10^{4}
743	10×10^{6}	33×10^{4}
744	2×10^{6}	1×10^{4}
745	6×10^{6}	16×10^{4}
746	15×10^{6}	1×10^{4}
747	1×10^{6}	8×10^{3}
748	16×10^{6}	19×10^{4}
749	8×10^{6}	24×10^{4}
750	3×10^{6}	5×10^{4}
751	13×10^{6}	41×10^{4}
752	8×10^{6}	11×10^{4}
753	6×10^{6}	1×10^{4}
755	4×10^{6}	17×10^{4}
756	2×10^{6}	4×10^{4}

Soil Micro Morphology

The Figures 3-5 shows scanning electron microscopic images of the investigated soils of the Caxiuanã and Santarém. The mineralogy was similar for all soils, consisting predominantly of kaolin in the clay fraction and quartz in the sand fraction, and they showed the predominance of the sandy texture (Figure 3). The presence of the bones and other material, such as wooden wool (cariapé), vegetal matter, and also sponges (cauxi) in ABE soil (Figure 4).

The Figure 5 shows material of the Santarém/Belterra. Have fine particles of the organic matter embedding pellets of lights yellowish kaolinitic clay particles. The topsoil showed no clear optical evidence of accumulation of organic matter.

Discussion

A large extent of soils in Amazon region is formed by yellow latosoils (yl). These soils have low chemical fertility but have anthropogenic activity of Indian population. They present ceramic and lithic artifacts and they have high fertility with high contents of OC, P, Ca, Mg, Zn and Mn. (Kern 1996, McCann et al. 2001, Schaefer et al. 2004, Costa & Kern 1999).

Table 3

Soil respiration (CO_2 flux), carbon (C-BMS) and nitrogen (NBM) microbial biomass of the ADE soil in the four places sites in Juruti, Pará State

Produtores	CO_2 flux	CBMS $cmol_c dm^{-3}$	NBMS
		0 – 5 cm	
Reinaldo	3,21 aA	125,68 bA	0,44bA
Felipe	1,56 cA	318,47 aA	0,41bA
Francisco	2,39 bA	113,68 bA	0,45 bA
Romão	2,88 abA	118,22 bA	0,58 aA
		5 – 10 cm	
Reinaldo	2,22 aB	46,02 aB	0,46 aA
Felipe	0,76 bB	66,42 aB	0,43 aA
Francisco	0,42 bB	53,91 aA	0,40 aB
Romão	1,18 abB	47,61 aA	0,49 aA
		10 – 20 cm	
Reinaldo	3,87 aA	152,30 bA	0,37 bA
Felipe	0,62 cB	371,60 aA	0,36 bA
Francisco	2,28 bA	122,85 bcA	0,42 abAB
Romão	0,42 cB	32,11 cA	0,48 aA

Letras minúsculas comparam produtores dentro de profundidades e maiúsculas comparam as profundidades com os produtores, pelo teste de Tukey a 5%.

Table 4

Soil respiration (CO_2 flux), carbon (C-BMS) and nitrogen (NBM) microbial biomass of the ADE soil in the two sites in Manaus City, Amazonas State

Sites	CO_2 $cmol_c dm^{-3}$	CBMS	NBMS
		0-5 cm	
P1P2	0,90 bA 1,79 aA	469,90 aA 303,80 aA	0,567 aA 0,490 aA
		5-10 cm	
P1P2	1,31 aA 1,17 aA	186,70 aA 233,86 aA	0,60 aA 0,67 aA
		10-20cm	
P1P2	1,27 aA 1,43 aA	186,74 aA 290,66 aA	0,61 aA 0,70 aA

Letras minúsculas comparam produtores dentro de profundidades e maiúsculas comparam as profundidades com os produtores, pelo teste de Tukey a 5%.

Studies of the soil micro morphology, chemical and biological show that high fertility of anthropogenic soils results from a favorable combination of mineral and organic components, making these soils highly enriched in exchangeable forms. The organic-mineral stabilization of soil organic matter showed that is mainly stabilized by chemic-sorption to mineral surfaces, as well as physical stabilization by entrapment into interior of aggregates (Glaser at al. 2004, Lima 2001, Ruivo et al. 2004).

Figure 1

Soil microbial population distribution in ADE sites from Caxiuanã - Pará State. Expressed in 10^3 CFU/g of soil

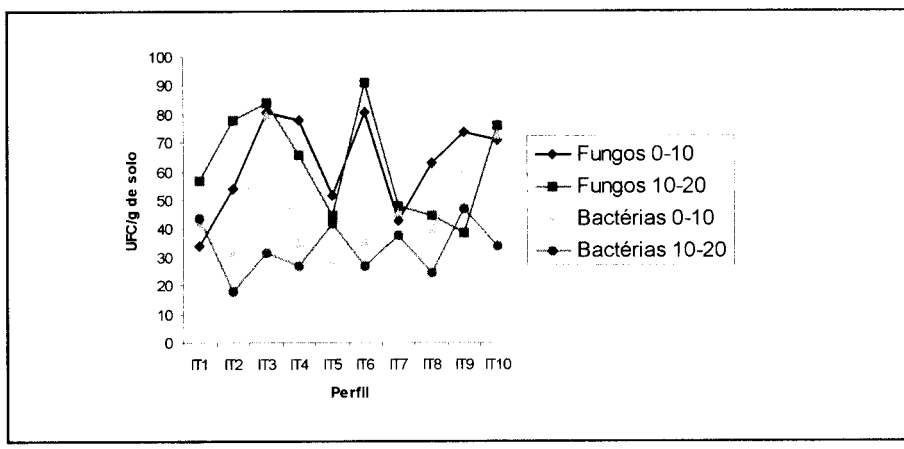

Figure 2

Soil microbial population distribution in ADE sites (B's; Z's) from Caxiuanã. - Pará State. Expressed in 10^3 CFU/g of soil

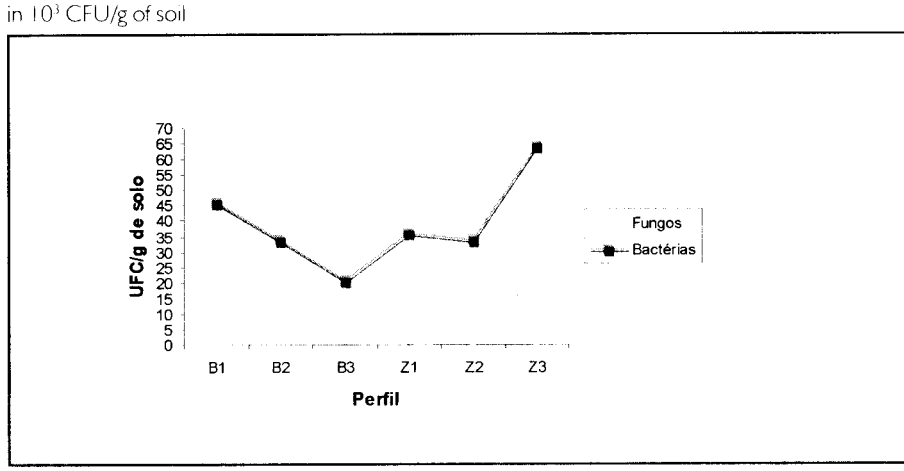

Figura 3

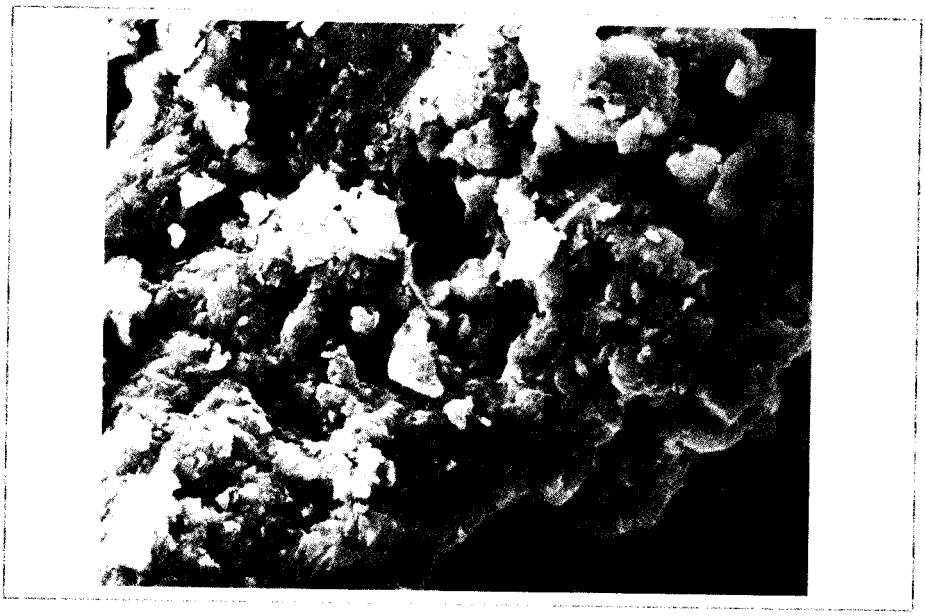

Figura 4

Micro morphology as tool to deduce processes of soil formation, natural transformation and human indicted processes (Ruivo & Cunha 2003). The characterization of the soil pore system and the types of soil structure is very important; the pores are large and more structured in ABE soils. The conservation of the structure micro aggregation is responsible for maintaining high levels of soil organic matter and available nutrients in ABE soils. Large amounts of non-consumed organic matter accumulated in the soil stimulate of microbial activity by these inputs may lead to accelerated mineralization of substances less resistant to degradation and enrichment of stable humic fractions rich and aromatic constituents (Zech et al. 1990).

This paper shows preliminary that the biodiversity is higher in the ABE than in Yellow Latosoil. The ABE soil is more aggregated than Yellow Latosoil. This factor facilitates soil aeration, roots distribution, water retention and movement. The ABE soils are used for subsistence agriculture, without fertilizers and distributed in small areas inside Amazon region. They maintain high values of P, Ca, Mg and OC. the high fertility of anthropogenic soils results from favorable mineral and organic factors (McCann 2001, Clement et al. 2004, Lovell et al. 2004).

The fire and ash have one important effect about soil fertility and increased microbiological activity adds colloidal-size organic components. This is verified in Roraima (Table 1) in the area altered Homoxi (YL after burn). The analysis of Amazon anthropogenic soils indicate that the alterations by human actions, such as incorporation of organic residues and the effects of fire in the superficial horizon influenced some of the chemical, physical and biological characteristics (Mc Cann et al. 2001, Glaser 1999). We believe that more occurrence of the production of the organic substances and micelles distribution in the ABE soils contributed for to maintain high biological activity and high nutrient retaining capacity.

In compared the micro biota of the Yellow Latosoil in the Dark Earths showed higher diversity, including a distinct more number of the fungal and bacteria genus, very occurrence of the actinomycetes, more occurrence of the organic substances and micelles distribution.

For this reason we need consider the microbial biomass of the ground that represents most of the active fraction of the organic substance and is important in the cycling of the nutrients, being able to be considered as reservoir of nutrients and energy, and therefore, with supplying potential of nutrients for the plants (Jenkinson & Ladd 1981). The carbon and the nitrogen used by the plants are derived from the decomposition of the organic substance, and this is entirely on the microbial biomass. The biggest importance of the microbial biomass, as source of nutrients for the plants, is on not only to C and N more also the biggest availability of the P (Marumoto et al. 1982).

In this way, the maintenance of the productivity of agricultural ecosystems depends, to a large extent, of the process of decomposition of organic substance therefore, of the microbial biomass of the ground (Gamma-Rodrigues 1999), that it functions as agent of transformation of the organic substance, in the cycle of nutrients and the flow of energy (Wardle 1993, De-Polli & War 1999, Martins et al. 1990).

Microbiota of the ground is an ecological pointer in the cycle of nutrients, therefore the microorganisms immobilize temporarily nutrient that could be availability its death after. The

activity and the size of the microbial community determine the intensity with that the processes biochemists happen. The amount and quality of the vegetal residues in the productive systems provoke alterations in the composition of the microbial community, influencing in the decomposition tax.

In this form, the systems of handling of the ground directly act in the persistence of the residues in the ground, reflecting in its physical, chemical and biological characteristics, in the size of microbial biomass e, consequentially, in the sustainability of agro ecosystems. Thus, the microbial biomass can be used to indicate the level of degradation of the ground, in function of the system of used handling (Doran & Parkin 1994), beyond organic carbon.

Besides those we can refer to vegetative cover, because it allows the reduction of thermal and water fluctuations, and so lowering remarkable oscillations in population density (Rovira & Davey 1974). We can still justify the bacteria population increase taking into consideration the possibility of an significant increase in the levels of both pH and moisture of the soil which benefits the bacteria growth whereas jeopardizes the fungi growth that tend to be abundant in acid soils (Alexander 1980).

In ADE the most part of these genera possess assuredly lignin and cellulose decomposers species (Roitman et al. 1991). This data is likely to contribute to the conclusion that the ADE soils are, presumably, more fertile than the average soils. Another aspect to point out is the differences found among bacteria counting from different ADE soils sites, which suggests that the sites' environmental heterogeneity play an important role in this result.

The counting techniques carried out in plates, as done in this study, attest the existence of only a minor portion of soil microorganisms, approximately 10% (Prescott et al. 1999). Only this fraction proved to be able to grow "in vitro" under laboratory environmental conditions. For this reason, the counting does not reflect the total amount of microorganisms present in the analyzed ADE soils. Because of that it is more accurate to consider the obtained results only as an indicator of microbial density in those kinds of soils, which means that the total microorganism's number could be 90% higher than the found amount portrayed in this experiment.