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Genetic and Functional Diversities of Microbial Communities in Amazonian Soils Under Different Land Uses and Cultivation

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1. Introduction

Amazonia is a natural region formed by the Amazon River Basin and covered by the largest equatorial forest in the world, covering an area of 6,915,000 km², of which 4,787,000 km² are in Brazil. Due to the large size and low population density, it is considered to be the best-well preserved Brazilian biome. Amazonian tropical forest soils are supposed to hold high microbial biodiversity, however the human impact has been extensive in the last decades, coupled with uncontrolled wood removal and the concomitant advancement of agricultural frontier (Fearnside, 2005).

Under the current scenario it is notorious the importance of Amazonia to the Brazilian ecosystem and even worldwide. Precisely because of this the images of slash-and-burn of the forest produce a strong impact on the public opinion. More than 60 million hectares were deforested. Of this total an estimated 35 million hectares were replaced by pastures for beef production, one million hectares were occupied with perennial crops, three million hectares with annual crops, and more than 20 million hectares support secondary vegetation called "capoeira" or fallow (Fig. 1).



Fig. 1. Conversion of forest (A) to well-managed pasture (B) and the fallow site (C) in the Amazon Forest.

What's occurring in the pastures at the Amazonia, as well in other tropical regions is the loss of the productive capacity after 4 to 10 years of use due to overgrazing, invasion of unpalatable weed species, loss of soil fertility and cultivation of inadequate grass species (Fernandes et al., 2002). It is estimated that 30 to 50% of pastures in the Brazilian Amazon are in advanced stage of degradation, giving rise to the fallow sites. In general, the establishment of pastures is done with simple technology and no use of fertilizers. Its maintenance depends almost exclusively on the nutrients contained in the ashes produced during burning of the original vegetation. Fallows also play an essential role for recovery of native species, as it reassimilates part of carbon and nitrogen that were released when slash-and-burn of native vegetation was used (Fernandes et al., 2002; Schroth et al., 2002).

The quality and soil fertility are defined from the point of view of some essential attributes that maintain the agricultural productivity, namely as: soil ability to promote plant growth, water supply and nutrient processing, efficient gases exchange in the atmosphere-soil interface and the activity of micro and macro organisms (Dilly & Nannipieri, 2001). In this context it is highlighted the role of soil microbial biomass (SMB), defined as the living portion of soil organic matter, excluding roots and larger organisms than, approximately, 5000 μm^3 (Cenciani et al., 2009).

In recent years many technological advances and the development of new and independent cultivation techniques led microbiologists to explore more precisely the "black box" of soil microbial diversity. This new knowledge is contributing to our better understanding of the distribution and abundance of soil microorganisms, the effect of community structure on ecosystem functioning, the effects of land use changes on microbial communities and hence in the ecosystem.

Traditional methods were usually based on specific cultivation media in laboratory conditions, in which only 1-3% of the soil microbes present conditions for growth. For this reason much research have been developed using generic properties, such as the microorganisms basal respiration, enzymatic activity, mineralization of soil organic matter, among others, that under controlled laboratory conditions represent rough estimates of the metabolic functions of microbial biomass, reflecting its physiology as whole soil community (Ananyeva et al., 2008).

Considered one of the most important “hot spots“ in the world, Amazonia has an important role in the discovery of new species of plants, animals and microorganisms, which may be important for the functionality of different ecosystems. However there are limited studies addressing the impacts of land use changes under the Amazonia microbial communities and their functions in the soil. Within this context bacterial and fungal communities, considered the most abundant groups of microorganisms in the soil, can act as important indicators of environmental stresses induced by the use of Amazonian soils.

Soil microbial diversity is usually assessed as species and genetic diversity rather than as structural and functional diversity. However, in terms of soil quality, these two last forms of diversity may be equally important due to the microorganism’s functional redundancy. The importance of functional and catabolic diversity lies in the fact that only based on changes in the genetic diversity; it is not possible to infer whether some functions of soils were lost or not (Mazzetto et al., 2008).

A soil with high redundancy of functions is probably able to maintain well-balanced its ecological processes, even under a disturbance. This approach, defined as resilience, refers to the buffering effects of external disturbances to the ecosystem. In a soil system the

reduction of microbial diversity can be an important indicator of the loss of resilience and, consequently, the soil quality. The abundance of some species of microorganisms seems not to be as important as the maintenance of their genetic and functional diversities. This is because the abundance reflects more immediately the short-term microbial fluctuation, while the diversity reveals the balance between the number of microorganisms and the functional domains in the soil (Kennedy, 1999; Lavelle, 2000).

The main objective of our chapter is to describe the relationship between the genetic and functional diversity approaches to study the microbial ecology and the impact of different land uses under soil microorganisms in Amazonia.

2. Microbial biomass in amazonian soils

The Amazon Basin covers almost 25% of South America. With about 7.5 million km², it extends into the territory of nine countries and accounts for 70% of tropical forests around the globe. Only in Brazil the total area is 5.1 million km² (Fearnside, 2005). Despite its great beauty and exuberance, the Amazon rainforest is found in soils of low fertility, while its maintenance depends on the cycling of nutrients from vegetation covering (Cenciani et al., 2009).

The quality and soil fertility are defined from the point of view of some essential attributes that maintain the agricultural productivity, namely as: soil ability to promote plant growth, water supply and nutrient processing, efficient gases exchange in the atmosphere-soil interface and the activity of micro and macro organisms (Dilly & Nannipieri, 2001). In this context it is highlighted the role of soil microbial biomass (SMB), defined as the living portion of soil organic matter, excluding roots and larger organisms than, approximately, 5000 μm³. The microbial biomass comprises the dormant and the metabolically active organisms in the soil; performing a primary role for maintenance and the products of microbial recycling are then absorbed by plant roots (Cenciani et al., 2009).

Soil quality or even "soil health" can be analyzed by the activity of microbial biomass, one of few active fractions of organic matter, sensitive to tillage and that can be quantified. Overall SMB comprises about 2-3% of total organic carbon in the soil, thus indicating it to be a sensible parameter to evaluate the quality of soils submitted to different management strategies, or to pollution impacts. The development of indirect methods for measurement of SMB such as the incubation-fumigation (IF) (Jenkinson & Powlson, 1976), the substrate induced respiration (SIR) (Anderson & Domsch, 1978), the content of ATP in microbial cells (Jenkinson & Ladd, 1981) and the extraction-fumigation (EF) method (Vance et al., 1987) facilitated the assessment of the SMB compartment.

Some studies previously carried out in chronosequences forest to pasture in Amazonia have shown that SMB is reduced after 3 years of establishing pastures, but their levels are raised in older pastures, and reach similar contents in the native forest. Several studies quantified the main elements (C, N, P, S) immobilized into microbial cells at different soil depths (Feigl et al., 1995 a,b; Fernandes et al., 2002; Cenciani et al., 2009).

Overall SMB reflects the contents of total organic matter, representing an efficient and sensitive parameter in assessing the quality of soils under different management or impacts of pollution. In Brazil, some studies realized in chronosequences forest to pastures in Amazonia have shown that microbial biomass is reduced in the early years (about three to five years), but increases in older pastures reaching levels similar to those of the native forest (Feigl et al., 1995 a,b; Fernandes, 1999). The ability of SMB to increase again in older

pastures, reaching values closer to the native forest suggests that the microorganisms of such soils have high resilience, or the capacity for growth and physiological activity, even after the impact of slash-and-burn of the native forest.

The stability of a system determines its ability to continue working under stress conditions, for both natural and those induced by human action (Orwin & Wardle, 2004). Since the microorganisms are the key players of the conversion of soil organic matter and the availability of nutrients, its resilience directly affects plant productivity and the stability of forest and agricultural ecosystems (Orwin & Wardle, 2005). For this reason it is essential to understand how microorganisms respond to environmental disturbances, as well as the factors involved in this response.

3. Diversity approach applied to soil microorganisms

Amazonian tropical forest soils are supposed to hold high microbial biodiversity, since they support by litter recycling one of the most luxuriant ecosystems. However anthropogenic practices of slash-and-burn, mainly for pasture establishment, induce deep changes in the biogeochemical cycles, and possibly in the composition and function of microbial species (Cenciani et al., 2009).

While the diversity of microorganisms in the soil is immense, only a very low percentage is cultivable (around 1%) under laboratory conditions. The limited range between the bacteria species, for example, hampers the detection by microscopy techniques. Additionally the methods of obtaining bacteria in culture medium are not very effective for its quantification, due to difficulties in reproducing the conditions that every species or groups require in their natural habitats (Felski & Akkermans, 1998). Estimates of the global diversity of fungi indicate that a small percentage is described in the literature, especially due to limitations found in techniques of cultivation to assess the diversity of fungi. Apart from this the lack of taxonomic knowledge hinders the identification of bacterial and fungal species found in the soil (Kirk et al., 2004).

The study of prokaryote diversity is extremely complex because the definition of species for these organisms is a question still open. Currently a prokaryotic species is regarded as a group of strains including the standard strain, characterized by some degree of phenotypic consistency showing 70% or more DNA-DNA homology and more than 95% similarity between the 16S rRNA gene sequences. In this context we highlight the importance of polyphasic taxonomy, which aims to integrate different datasets and phenotypic, genetic and phylogenetic information about the microorganisms (Gevers et al., 2005).

With the advance of molecular biology it became possible to identify bacteria, fungi and other microorganisms in the soil and plants without need to isolate them. One of cultivation-independent molecular tool that has often been used to analyze the diversity and dynamics of microbial populations in the environment is the polyacrylamide gel electrophoresis in denaturing gradient (DGGE). The DNA is extracted and purified and only a fragment of the rRNA gene is amplified by the polymerase chain reaction (PCR). The amplification products are analyzed by gel electrophoresis, which allows the separation of small PCR products, commonly up to 400 bp according to their contents of guanine plus cytosine (G+C) Consequently, the fingerprinting pattern is distributed along a linear denaturing gradient (Muyzer & Ramsing, 1995; Courtois et al., 2001; Cenciani et al., 2009).

3.1 *Fungi* diversity assessed by PCR-DGGE

The role of fungi in the soil is complex and fundamental to maintain the functionality of the biome. Fungi play an active role in nutrient cycling and develop pathogenic or symbiotic associations with plants and animals, besides interacting with other microorganisms (Anderson & Cairney, 2004).

Working with soils in the Amazonia, Monteiro et al. (2007) described the changes in the genetic profiling of soil fungal communities caused by different land use systems (LUS): primary forest, secondary forest, agroforestry, agriculture and pasture. The author conducted her study in the following sequence: DNA extraction - total DNA was extracted using the Fast DNA kit (Qbiogene, Irvine, CA, USA), according to the manufacturer's instructions; PCR - a fragment of the 18S rRNA gene (1700 bp) of fungi was amplified by PCR according to Oros-Sichler et al. 2006; DGGE – amplicons were separated on an acrylamide gel containing bisacrilamide and a linear gradient of urea and formamide (Fig. 2).

Diversity Database program (BioRad) was used to determine the richness of amplicons. The non-metric multidimensional scaling (NMDS) tool was used to determine the effect of land use changes under the fungi communities through the PRIMER 5 program (PRIMER-E Ltd., 2001).

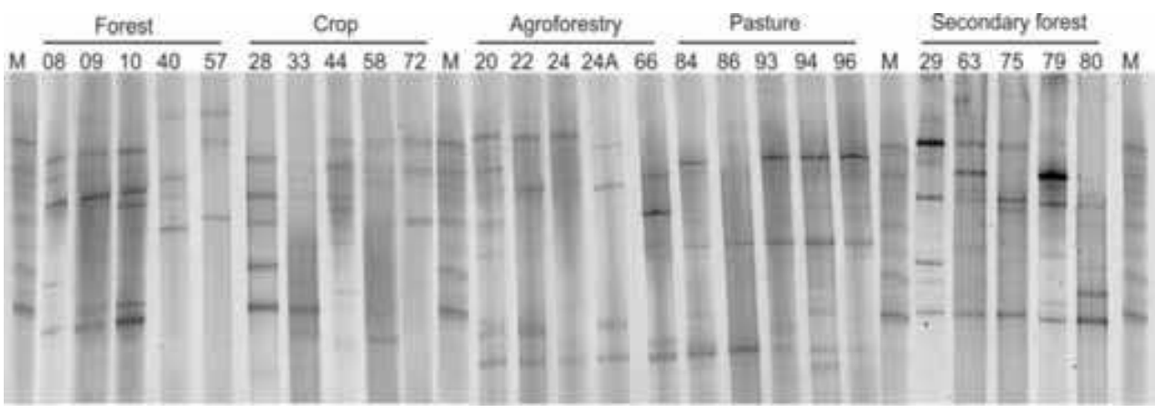


Fig. 2. DGGE gel of 25-38% urea and formamide, generated by separation of 18S rRNA gene fragments amplified from samples of natural soils under different LUS. M – molecular marker.

The DGGE of the 18S rRNA gene combined with NMDS statistic tool showed the presence of distinct communities in each of the areas analyzed, with the presence of single bands. Results indicated the dominance of specific fungal groups in every treatment, especially in the area converted to pasture, distant from the other systems of land use (Fig. 2).

Following this pattern the authors asserted that the banding profile generated by DGGE represent fungi communities from different soils, and were shown to be more similar among samples from the same system of land use than among samples of different systems of land use. However the clustering of samples through NMDS showed that there is a tendency for samples from pasture be different of the other sites, which are closest relatives among them (Fig. 3). Finally the results obtained by the authors show that changes in the land use affected the community structure of soil fungi; as well it is also possible that the type of vegetation covering has a key role in such changes (Monteiro et al., 2007).



Fig. 3. Non-metric multidimensional scaling (NMDS) of 18S rRNA gene amplicons from soils of the Amazon forest under different systems of land use: secondary forest, forest, crop, agroforestry and pasture – stress 0.13 (A); and secondary forest, forest, crop, agroforestry and pasture – stress 0.15 (B).

Although molecular fingerprinting approaches such as cloning and sequencing are being used increasingly for evaluation of fungal communities, there are scarce studies reaching the diversity of fungi in soils of native forests, and in the same soils but impacted by agricultural management. Within this context changes in the genetic profile of fungi according to each system of land use, and the environmental stress can provide valuable information for the sustainable management of forest soils (Monteiro et al., 2007).

3.2 *Bacteria* diversity assessed by PCR-DGGE

Advances in molecular approach such as the DNA profiling through PCR-DGGE can also provide information regarding the composition of bacterial populations in soils. Cenciani et al. 2009 examined how the clearing of Amazonian rainforest for pasture and the seasonality affected the diversity of *Bacteria* domain. The aim of this study was to assess the extension that land use changes in Amazonia had on the structure of *Bacteria* domain.

According to Cenciani et al. (2009) field works were developed at Nova Vida Ranch (62º49`27``W; 10º10`5``S), in the central region of Rondonia state (Fig. 4). The predominant soil is classified as Argissolos in the Brazilian classification system (Empresa Brasileira de Pesquisa Agropecuaria - EMBRAPA, 2006) and as Ultisols (Kandiuldults) in the US soil taxonomy. It is a representative soil of Amazonian basin covering almost 22% of the Brazilian Amazonian basin. The Nova Vida Ranch covers an area of approximately 22.000 ha, consisting of a mixture of native forest and pastures of different ages. Pastures were established with no mechanical machinery nor chemical fertilization and soil acidity correction. Wood weeds were controlled by cutting the aerial part, removing the residues and burning them to reduce volume and incorporate the ashes into the soil (Feigl et al., 2006).

A sequence was chosen at Nova Vida: (1) a 3-ha plot of native forest, (2) a well-established pasture of 20 years (*Brachiaria brizantha* and *Pannicum maximum*), and (3) a fallow site (Fig. 1). The botanical composition of fallow includes 15-18% of woody species (*Tabebuia* spp., *Erisma uncinatum* and *Vismia guianensis*), 12% of Babaçu palm (*Orbignya phalerata* Mart), herbaceous weeds 4-11%, and 63.5% of a mixture of *Brachiaria brizantha* and *Pannicum maximum* (Feigl et al., 2006). Soil samples were taken at surface layer (0-10 cm) in the rainy season and 6 months later, in the dry season.

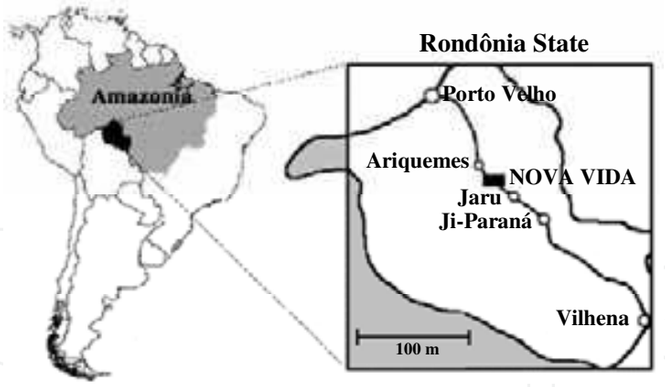


Fig. 4. Map of the study site located in Rondonia State.

Total soil DNA extraction and PCR products were generated according to conditions described by ∅vreas et al., 1997. PCR products (300 ng) were resolved using DGGE to provide the molecular profiles of bacterial communities. The structure of similarity for *Bacteria* was generated from binary data. Dendrograms representing hierarchical linkage levels were constructed based on the Euclidean distance coefficient using Systat 8.0 software.

As expected PCR with specific primer sets including the forward primer coupled with a GC clamp resulted in a single 180-bp fragment. PCR products were separated by DGGE to assess the qualitative bacterial composition. Some groups of bands, exemplified as I to VI, were chosen to better compare similar and/or different band profiles (Figs. 5 and 6).

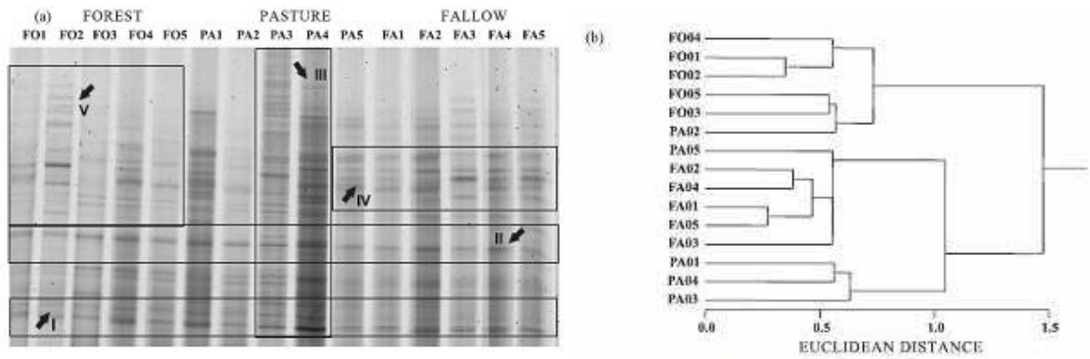


Fig. 5. DGGE (a) and cluster analysis (b) of the 16S rRNA gene in Amazonian soil samples, collected in the wet season.

In the Figure 5a (wet season), some bands were found in all soil replicates (I, II). It means that they were present in the DNA extracted from each sample and it indicated the presence of the same bacterial community in the three sites. Pasture was characterized by the presence of band patterns concentrated in PA3 and PA4 (III), and IV is a band profile found in the fallow and in the PA5 replicate of pasture. Forest contained replicates with high variability of band patterns; therefore FO2 contained more bands than the others (V).

DGGE profiling in the dry season (Figure 6a) revealed more visible differences in the bacterial structure among the sites than in the wet season. Band patterns I and II were presented in almost all samples, except FA1 to FA4. Group III represented bands common to

pasture and fallow, while IV and V were bands specific to replicates FO1 to FO4 and FO1, respectively. VI was a particular banding pattern from pasture. It was not found a band profile presented specifically in the fallow site. Independently of sampling period, similar bands were found among the sites; as well each site had its own particular bands along DGGE profile.

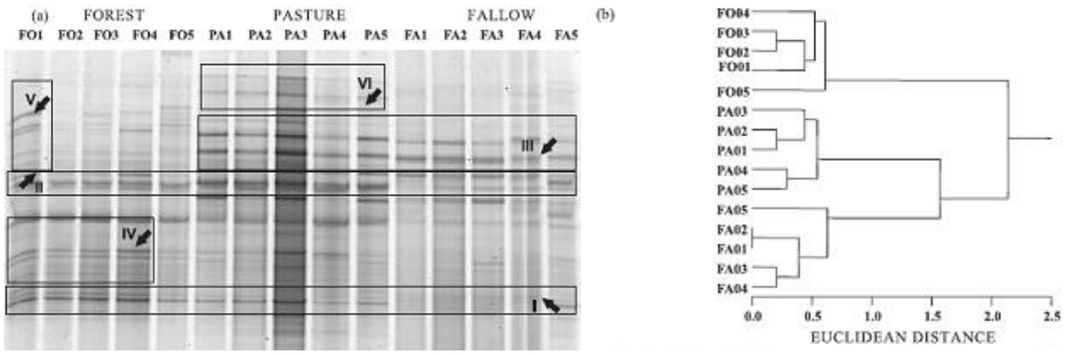


Fig. 6. DGGE (a) and cluster analysis (b) of the 16S rRNA gene in Amazonian soil samples collected in the dry season.

In the cluster analysis of PCR-DGGE products, the three sites clustered at 65% level of similarity for both wet and dry seasons. Data presented in Figure 5B shows that, in the wet season, bacterial communities were separated in three clusters, except PA2 replicate that tended to group together forest cluster; whereas PA5 replicate fell into the fallow cluster. In the Figure 6B, the effect of low water content plus history of soil use contributed to separate completely the bacterial populations from each site during the dry season. The variation in the composition of microbial community DNA between replicate soil samples was found to be as great as the variation between treatments in field based studies. The reasons for such variability are not clear, however it is likely that are attributable to the effect of soil chemical attributes plus the contents and composition of organic matter (Clayton et al., 2005; Ritz et al., 2004).

According to the authors the DGGE profiling revealed lower number of bands per area in the dry season, but differences in the genetic diversity of bacterial communities along the sequence forest to pasture was better defined than for wet season. The few research works using molecular approaches to investigate the diversity of microorganisms in Amazonia have shown that, in fact, a tiny fraction of their microbial diversity is known (Cenciani et al., 2009).

3.3 Other molecular tools applied to microbial diversity in amazonian soils

Soil microbial diversity is still a difficult field to study, especially due to the several limitations of techniques. Since 95-99% of organisms cannot be cultivated by culture based-methodologies, the microbial diversity of soils shall be assessed by molecular biology techniques (Elsas & Boersama, 2011).

New DNA and RNA sequencing techniques provide high resolution information, especially using depth sequencing of metagenomic samples. Most of times a high amount of the obtained sequences are related with unknown genes or unknown organisms, involving a high cost per sample. Since soils imply in most of times in high spatial variability, which means high number of samples and replicates, fingerprinting techniques are recommended prior to sequencing in order to reduce costs for the high resolution techniques.

The first study of microbial diversity in Amazon soils using molecular techniques, by means of clone library, showed a high prokaryotic diversity (Borneman & Tripplett, 1997). Analyzing 100 sequences, differences between mature forest and pasture were detected, and about 18% of sequences were related to unknown Bacteria. A decade after, analyzing 654 clones similar results were detected in other study site, in which 7% of sequences could not be classified in any bacterial phyla (Jesus et al., 2009). In both studies land use changes was an important factor, and the unknown species were surveyed showing that depth sequencing should be used to better characterize the Amazon soils.

The most popular techniques for soil microbial communities fingerprinting are DGGE and the terminal restriction fragments length polymorphism (T-RFLP), which should be complemented by sequencing information to provide an overview of the study sites. Such techniques consist in extraction of nucleic acids from the soil samples; followed by amplification by PCR, aiming to target specific microbial groups according to the primers chosen (i.e. a universal primer for 16S rRNA gene will give a general prokaryotic overview of the samples). After PCR the amplicons should be analyzed by denaturizing gel separation (DGGE) or digestion with restriction enzymes and analysis of the dye labeled fragments (T-RFLP), or DNA sequencing. In turn metagenomics techniques allow sequencing without preview amplification by PCR and other techniques to be considered (Elsas & Boersama, 2011).

T-RFLP consists in a PCR using dye labeled primers followed by a digestion with restriction enzymes, purification and reading in a DNA sequencer. The PCR amplifies a specific gene (mainly the 16S rRNA gene for prokaryotic diversity), and the restriction enzymes fragment the PCR products according to its polymorphism. The sequencer separates the fragments by length reading them in an electrophoresis run. So the presence of distinct fragment sizes found in different soil samples allows the diversity separation among them (Jesus et al., 2009). Clone libraries consist in cloning the PCR amplicons into bacterial vectors, followed by DNA sequencing. Since the PCR from environmental samples amplify different DNA sequences of different organisms at the same time, cloning technique allows the separation of amplicons and the sequencing of individual sequences (Borneman & Tripplett, 1997). Different studies using other molecular approaches to access the diversity of Amazon soils (Table 1) are described below.

In Western Amazon a T-RFLP analysis of the bacterial communities showed how it was influenced by soil attributes correlated to land use (Jesus et al., 2009). Community structure changed with pH and nutrient concentration. By DNA sequencing, bacterial communities presented clear differences among the different sites. Pasture and one of crops presented the highest diversity. Secondary forest presented similar diversity with the community structure of the primary forest, showing that bacterial community can be restored after agricultural use of the soils. Using the automated ribosomal intergenic spacer amplification (ARISA) technique distinct microbial structures were also observed between agricultural and forest soils (Navarrete et al., 2010). Seasonal changes in the two different years of sampling and distinct band patterns were observed for fungal, bacterial and archaeal richness.

Different patterns between Terra Preta soil (Dark Earth or Anthrosols) and an adjacent soil were observed in the Southwestern Amazon using 16S rRNA gene sequencing (Kim et al., 2007). *Acidobacteria* were predominant in both sites but 25% greater species richness was

observed in the Antrosol. In other study in three Dark Earth sites near Manaus, "Lago Grande", "Hatahara" and "Açutuba", a cultivable bacteria survey showed a higher richness in Antrosols than in the adjacent soils (O'Neill, 2009). Several bacteria were isolated using rich media or soil-extract media and genetic groups were separated by RFLP. By sequencing, *Bacillus* was the most abundant genera.

Main Aim of the Study	**Technique(s)**	**Localization (States of Brazil)**	**Reference**
Compare *Bacteria* diversity in forest and pasture soils	Clone Library	Paragominas, Para (2°599S; 47°319W)	Borneman & Tripplett., 1997
Investigate Dark Earth bacterial diversity	Clone Library	Jamari, Rondonia (8°45'0S; 63°27'0W)	Kim et al., 2007
Compare Bacterial communities in Anthrosols and adjacent soils	Bacteria isolation + RFLP + Sequencing	Manaus, Amazonas (3°08′S; 59°52′W)	O'Neill, 2009
Investigate land use impact on soil *Bacteria* structure	T-RFLP + Clone Library	Benjamin Constant, Amazonas (4°21S,69°36W; 4°26S,70°1W)	Jesus et al., 2009
Compare Anthrosols with adjacent soils	DGGE followed bands Sequencing + T-RFLP	Manaus, Amazonas (3°08`S; 59`52'W)	Grossman et al., 2010
Investigate microbial communities in agricultural systems	ARISA + T-RFLP + Pyrosequencing	Benjamin Constant, Amazonas (4°21S, 69°36W; 4°26S,70°1W) + Iranduba, Amazonas (03°16'28.45"S; 60°12'17.14"W)	Navarrete et. al., 2010
Land use in *Archaeal* and *amo*A structures in Dark Earths	T-RFLP + Qpcr + Clone Library	Manaus, Amazonas (from 02°01′52.50″S, 26′28.30″W; to 03°18′05.01″S, 60°32′07.38″W)	Taketani, 2010
Investigate Archaeal structure in a wetland soil	Clone Library + methanogenic bacteria isolation	Santarem, Para (02°23'20"S; 54°19'39.5"W)	Pazinato et al., 2010
Investigate the influence of different land uses on the bacterial structure of Cerrado and Forest Soils	T-RFLP	Sinop (Tropical Forest - S120553.3W; 552846.0) and Campo Verde (Cerrado - S 151588.8; W 550700.0), Mato Grosso	Lammel et al., 2010

Table 1. Diversity studies using other molecular biology techniques in Amazon soils

Grossman et al. (2010) studying the three same Dark Earths sites, including one additional site, "Dona Stella", and using different molecular techniques also found difference among the samples.. T-RFLP of the 16S rRNA genes provided clear distinction between the two types of soils, and the same result was observed using DGGE and 16S rRNA sequencing. While T-RFLP provided a good fingerprinting between Anthrosols and Adjacent soils, 16S rRNA sequencing provided better resolution of the changes, indicating *Verrucomicrobia* as an important group to the Anthrosols, *Proteobacteria* and *Cyanobacteria* for Adjacent soils; while *Pseudomonas*, *Acidobacteria* and *Flexibacter* were found in both sites.

Studying the "Hatahara" site, differences in bacterial communities were also observed among Amazonian Dark Earth, black carbon and an adjacent oxisol by T-RFLP (Navarrete et al., 2010). By pyrosequencing it was shown that the most predominant phyla were *Proteobacteria, Acidobacteria, Actinobacteria* and *Verrucomicrobia*. About one-third of the sequences corresponded to unclassified *Bacteria*. For archaeal structure comparison by T-RFLP the soil attributes were more important than the type of soil, if it was Terra Preta or adjacent soils (Taketani, 2010). DNA sequencing showed that *Candidatus spp.* was the most abundant genera in both types of soils. An *amo*A clone library showed differences among the sampled sites, but also did not show differences between Terra Preta and the adjacent soil.

Using T-RFLP of bacterial 16S rRNA, distinct patterns were observed among biomes and land uses in the Southwestern Amazon (Lammel et al., 2010). Southwestern Amazon is divided in two mainly biomes, Tropical Forest and Cerrado (Brazilian Savanna). Over the last three decades these natural vegetations have been converted to pasture and agriculture. Land use was the most important factor to distinguish the bacterial communities, and it was correlated with the soil chemical changes: pH - due to liming and chemical fertility - due to fertilizers application. Pristine Tropical Forest and Cerrado formed distinct clusters, but they were more similar to each other than in relation to pasture or soybean field (Fig. 7).



Fig. 7. Different land uses (native forest, native cerrado, soybean field and pasture) studied by Lammel et al. (2010).

In Eastern Amazon wetland soils Archaeal community was characterized by 16S rRNA gene libraries and by isolation of methanogenic Archaea (Pazinato et al., 2010). Archaeal diversity decreased with depth and the most of sequences belonging to *Crenarchaeota, Methanosarcina* and *Metahnobacteriam* genera were isolated from the sites.

These different techniques showed a high microbial diversity on Amazon soils. Fingerprinting techniques, such as T-RFLP and ARISA, were sensitive tools to detect difference in the microbial structure among the different sites and land uses. However only DNA sequencing provided a better resolution of the diversity, i.e. identify taxonomic groups and report unknown *Bacteria* that probably belong to new taxonomic groups. These pioneer studies showed, in general, that diversity does not decrease from pristine vegetation to agricultural uses, but the structure of microbial community as a whole is affected by land use changes. They can be restored after stopping the soil cultivation followed by secondary forest growth. The Amazon region is a "hot spot" regarding the soil microbial diversity.

3.4 Arbuscular mycorrhizal fungi

Arbuscular mycorrhizal fungi (AMF) are also an important microbial group in soil, since they can form symbiosis with most of the plants, contributing to plant health and nutrition. AMF is beneficial to tropical plants and presents potential influence on soil processes and plant diversity, increasing the interest For studying this group this group, especially in Amazon where little is known about them (Stürmer & Siqueira, 2010).

Most of AMF studies consist on identification of its spores from soil samples. Since AMF produce spores significantly bigger than the other fungi species, it is possible to separate them from soil samples by sieve and centrifugation in a sucrose gradient. Up to now, the studies in Brazilian Amazon were made using this approach (Leal et al., 2009; Mescolotti et al. 2010; Stürmer and Siqueira, 2010).

In Southwestern Amazon an AMF study compared three land uses: native vegetation, soybean fields and pastures, in two regions: Sinop (Forest) and Campo Verde (Cerrado), both in Mato Grosso State, Brazil (Mescolotti et al., 2010). Comparing Forest with Cerrado different patterns were observed. The largest amount of spores was found in soybean fields in the Forest region, and the number of spores was the same for the three land uses in the Cerrado region. *Glomus* spp. was the most common specie found (Fig. 8.).

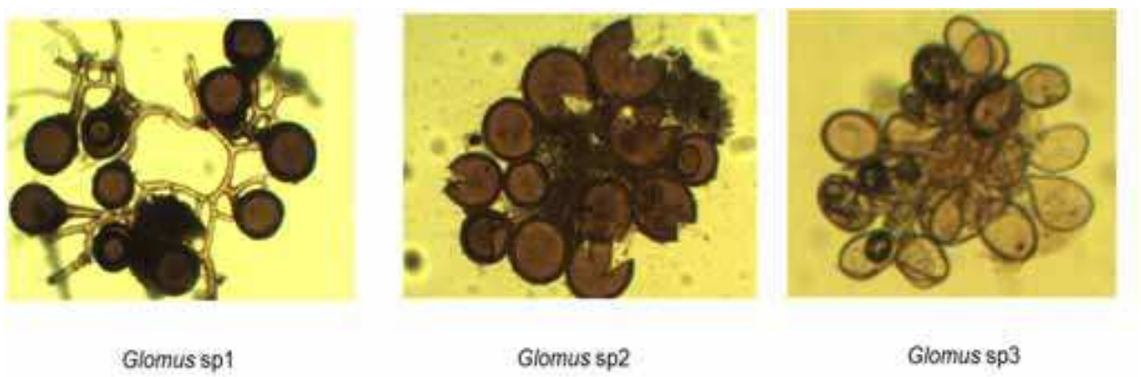


Fig. 8. AMF surveyed in Southwestern Amazon. *Glomus* spp was the most common (Mescolotti et al., 2010).

In Western Amazon different AMF patterns were observed in different land uses (Stürmer & Siqueira, 2010). A total of 61 AMF morphotypes were recovered and 30% could not be classified as known species. *Acaulospora* and *Glomus* were the most common genera identified in the sites and higher AMF richness values were found in agriculture and pasture sites, than in the pristine areas. AMF patterns were also influenced by land use in a survey using different trap cultures in the same region (Leal et al., 2009). Among all trap plants and land uses, a higher number of spores were found in pasture and young secondary forest. In total 24 AMF species were recovered. *Acaulospora* spp. (10 species) was the most common genera followed by *Glomus* spp (5 species). Both studies showed that in Amazon soils the land use change from pristine vegetation to pasture and crops did not reduce the AMF diversity and probably new AMF species were found.

3.5 Catabolic diversity profile

Catabolic diversity profile (CDP) is a method aiming to measure the similarity of the catabolic functions of microbial communities in different soils or changes in the same soil

under different treatments or land uses, or yet the intensity of respiratory responses to a range of substrates tested (Table 2). The richness (variety) of catabolic diversity is given by the total number of substrates that could potentially be used by the microbial community. The higher is the index of similarity, the greater is the diversity of microbial population; as it is maintained the ability of soil microorganisms to give an intense respiratory response to all substances (substrates) tested. With a reduction of microbial diversity, it is lost some species able to metabolize certain functional groups, and with it, the ability of the system to react (resilience) in the form of CO_2 emission decreases. The lower is the index of similarity; the lower is the diversity of microbial population (Van Heerden et al., 2002).

Substrates	Amine	Carbohydrate	Aminoacid	Carboxilic Acid
Glutamine	X			
Glucosamine	X			
Glucose		X		
Manose		X		
Arginine			X	
Asparagine			X	
Glutamic Acid.			X	
Histidine			X	
Lisine			X	
Serine			X	
Citric Acid				X
Ascorbic Acid				X
Glucomic Acid				X
Fumaric Acid				X
Malonic Acid				X
Malic Acid				X
Ketoglutaric Acid				X
Ketobutiric Acid				X
Pantotenic Acid				X
Quinic Acid				X
Succinic Acid				X
Tartaric Acid				X

Table 2. Substrates used in the catabolic diversity profile of soil microorganisms.

The two most common methods to measure the utilization of substrates by microorganisms are Biolog (Garland & Mills, 1991; Zak et al., 1994) and the respiratory response to addition of substrates, known as substrate induced respiration (SIR) (Degens & Harris, 1997; Degens et al., 2001). The authors claim that these techniques are sensitive enough to distinguish changes in the catabolic diversity that occur over short periods of time, as well as large differences that occur in the soil after a few years (Graham & Haynes, 2005). The main substrates used for SIR analysis are shown in Table 2. The diverse substrates are dissolved in 2 ml of solution for each equivalent of 1g dry soil and incubated in sealed bottles. The flow of CO_2 for each sample is usually measured in an Infra-Red Gas Analyser (IRGA), after incubation of bottles for 4 hours at 25^{o}C.

Few studies have been carried out in the Amazon region. Among these is the work of Mazzetto et al. 2008. This research evaluated the possibility to check whether there are

catabolic patterns in the Amazon soils under agricultural cultivation, native forest and pasture. A total of 60 areas were chosen distributed as: 20 native forest, 20 agricultural lands and 20 pasture sites in the regions of Mato Grosso and Rondonia, which are part of the Brazilian Amazon.

At first analyses were performed only in the native areas, which could be separated in Amazon rainforest, Cerrado and Cerradão. The low catabolic response obtained in the Cerrado soils may be linked to the frequent firing process that this biome suffers (Fig. 9). According to Arocena & Opio (2003), fire has a major impact on the physical (aggregate stability, clay content) and chemical (pH) soil properties, with significant influence on the microbial biomass. According to Hart (2005) fire alters the structure of microbial biomass, this being a selection factor in areas exposed to periodic events. Campbell et al. (2008) demonstrated in their studies that the use of carbonated substrates decreases with burning of area, suggesting a lower resistance/resilience of the microbial community. Among the substrates that can be influenced by burning of vegetation is arginine, which has a low response in Cerrado and Cerradão soils. The use of arginine in the microbial metabolism requires the presence of deaminase arginine enzyme, which is inhibited by fire.

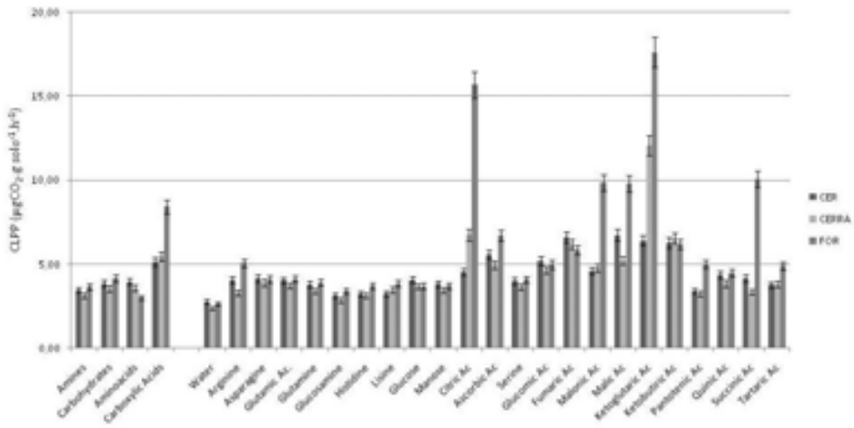


Fig. 9. Catabolic profile of soil microbial biomass in native areas: Cerrado (CER), Cerradão (CERRA) and Forest (FOR).

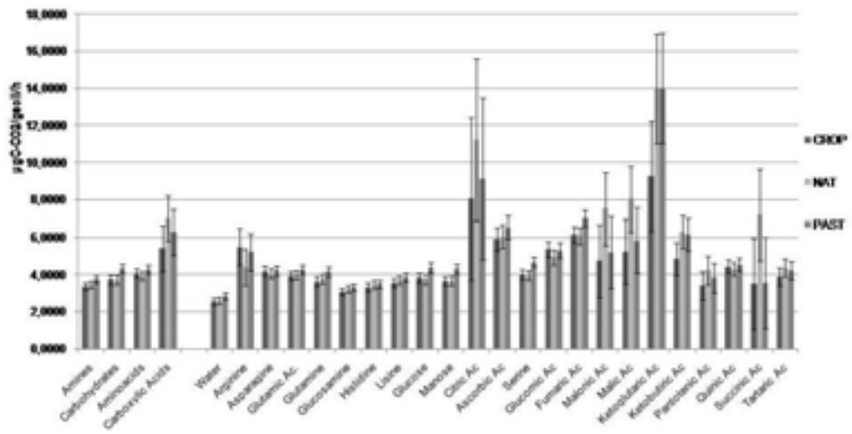


Fig. 10. Catabolic profile of soil microorganisms in agricultural areas (CROP), native areas (NAT) and pasture areas (PAST).

Regarding the disturbed areas analysis were realized aiming to characterize the diversity of soil microbial biomass at these sites (Fig. 10), and to check the possible separation of the areas through multivariate statistical analysis (Fig. 11).

Soils under pasture had significant catabolic responses to amine and carbohydrate, and individually to the substrates glutamic acid, glutamine, glucose, mannose, serine and fumaric acid. In contrast soils under native vegetation had significant responses to malonic acid, malic acid and succinic acid. Soils under agriculture use did not show significant responses to any substrate examined, however they showed expressive responses to the aminoacids group, but not statistically different from the pasture soil (Fig. 10).

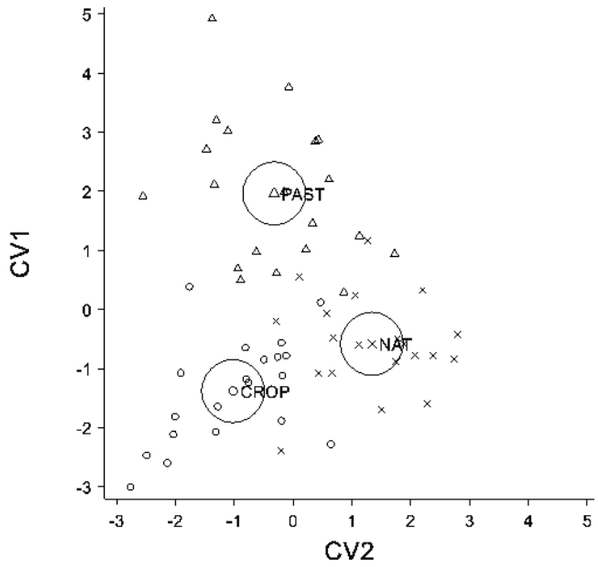


Fig. 11. Canonical analysis of the catabolic profile of microorganisms. Coefficient variation 1 (CV1) explained 67.50% of variability, while CV2 explained 32.50%. (Δ) Pasture, (○) Agricultural Areas, (x) Native Areas.

The canonical analysis showed that datasets related to CDP had great success in distinguishing the three land uses analyzed (Fig. 11). CV1 explained 67.5% of the variability observed, separating pastures from native areas and agriculture. Averages of native and agriculture areas were negative (-1.38 and -0.58, respectively) for CV1, while the average of pasture was positive (1.96). Asparagine, histidine and quinic acid with highly negative values were closely tied to native areas and agriculture, while glutamic acid and glucosamine had great representation in relation to pasture. CV2 explained 32.5% of the variability observed, separating native areas from agriculture and pastures. The average of native areas for the second axis was positive (1.34), while those of agriculture and pastures were negative (-1.02 and -0.32, respectively). The main substrates that provided this separation were serine and quinic acid, which showed negative values (linked to pasture and agriculture), and the tartaric acid, considered the more representative substrate related to native areas.

Among the major substrates involved, serine is documented as present in root exsudates (Bolton et al., 1992), quinic acid is a component of plant tissues (Gebre & Tchaplinski, 2002), and tartaric acid is one of main intermediary compounds of the Krebs cycle, in the basic metabolism of aerobic microorganisms (Tortora et al., 2005).

When only one ecoregion (Alto Xingu) was selected for analysis results of the CDP approach was even more significant (Fig. 12). CV1 explained 66.5% of the variability, separating native areas (-7.87 - negative score) of areas under agriculture and pasture (4.33 and 0.49 – positive scores, respectively). The main substrates involved in such axis were: succinic acid and malonic acid, with negative values. With positive values quinic acid and glucose also contributed to the separation observed. CV2 explained the remaining 33.5% of the variability, separating areas under pasture (4.84 – positive score) of native and agricultural areas (-2.04 and -2.65 – negative scores, respectively). Among the major substrates in this axis are highlighted asparagine and tartaric acid showing negative values, while lysine and pantothenic acid had positive values (Fig. 12).

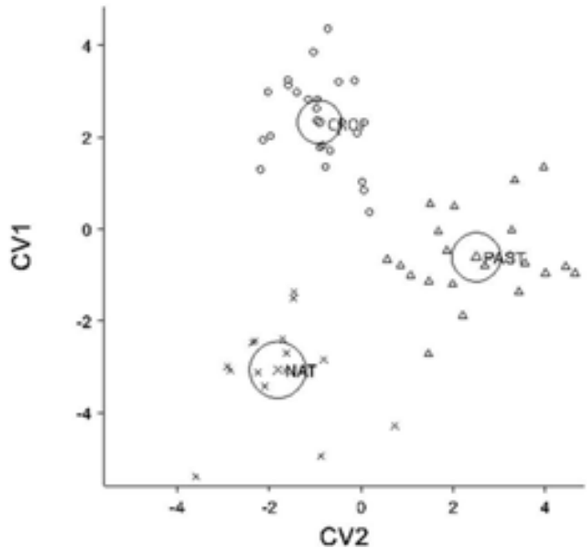


Fig. 12. Canonical analysis of the catabolic profile of microorganisms in the Alto Xingu ecoregion. CV1 explained 66.5% of variability, while CV2 explained 33.50%. (Δ) Pasture, (○) Agricultural Areas, (x) Native Areas.

Taking into account only data corresponding to the agricultural areas present in the database, we could distinguish areas under perennial crops, tillage and conventional tillage. By means of discriminant analysis the reallocation of data was performed in order to observe if datasets was homogeneous among the land uses analyzed. Data from areas under conventional tillage were relocated with 70% success, while data from conventional tillage and perennial cultivation showed higher percentage (98% and 100%, respectively). The same analysis was performed for pasture data that could be reallocated according to the following classification: typical pasture (100% success), improved pasture (95% success) and degraded pasture (91% success). This high percentage of reallocation of data shows that the microbial communities analyzed by CDP have high correlation with the use of land deployed. According to Mazzetto et al. 2008 the application of substrate induced respiration was efficient in distinguishing the land uses. The composition of microbial community revealed, through CDP approach, a close relationship with vegetation cover, regardless of climatic factors or the soil type.

As highlighted by Tótola & Chaer (2002) and San Miguel et al. (2007), the importance of functional and catabolic diversity lies in the fact that only based on changes in the genetic

diversity it is not possible infer whether some functions of soil were lost or not. The physiological profile of microbial community allows accessing the metabolic capacity of the microbial biomass as a whole, through tests realized with specific carbon sources defined in the laboratory.

4. Conclusion

Soil microbial diversity is still a difficult field to study, since 95-99% of organisms cannot be cultivated by culturing methodologies. The most popular techniques for soil microbial communities fingerprinting are DGGE and T-RFLP, which should be complemented by sequencing information to provide an overview of the study areas, especially those with high spatial variability that requires the collection of a high number of samples and replicates. New DNA and RNA sequencing provide high resolution information especially using depth sequencing of metagenomic samples.

Using DGGE, T-RFLP and other approaches, it has been clear that land use changes influenced significantly the diversity and structure of microbial communities in the Amazonian soils. Data available of DNA sequencing provided a high resolution view pointing changes of specific microbial groups and also the high quantities of unknown microorganisms. Catabolic diversity profile was efficient in distinguishing the land uses. The composition of microbial community revealed, through CDP approach, a close relationship with vegetation cover, regardless of climatic factors or the soil type.

Land use changes modify the genetic structure of microbial communities in the Amazonian soils, but they do not reduce the diversity in the areas affected by deforestation and conversion for pasture and crops, in comparison with the native areas. Also many new species are to be discovered in such areas.

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6. Appendix

Acronyms and Abbreviations

AMF - Arbuscular Mycorrhizal Fungi
ARISA – Automated Ribosomal Intergenic Spacer Amplification
CAPES – Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior
CDP – Catabolic Diversity Profile
DGGE – Gel Electrophoresis in Denaturing Gradient
EF – Extraction-Fumigation
EMBRAPA – Empresa Brasileira de Pesquisa Agropecuaria
FAPEMIG – Fundacao de Amparo a Pesquisa do Estado de Minas Gerais
FAPESP – Fundacao de Amparo a Pesquisa do Estado de Sao Paulo
IF – Incubation-Fumigation

IRGA – Infra-Red Gas Analyser
LUS – Land Use Systems
NMDS – Non-Metric Multidimensional Scaling
PCR – Polymerase Chain Reaction
RFLP – Restriction Fragments Length Polymorphism
SIR – Substrate Induced Respiration
SMB - Soil Microbial Biomass
T-RFLP – Terminal Restriction Fragments Length Polymorphism

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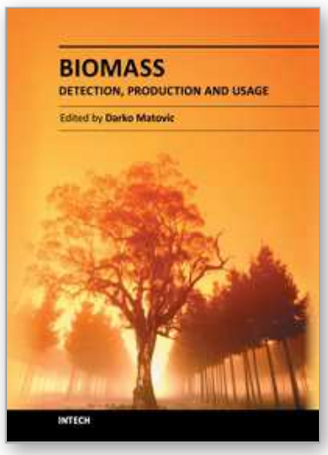
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Biomass has been an intimate companion of humans from the dawn of civilization to the present. Its use as food, energy source, body cover and as construction material established the key areas of biomass usage that extend to this day. Given the complexities of biomass as a source of multiple end products, this volume sheds new light to the whole spectrum of biomass related topics by highlighting the new and reviewing the existing methods of its detection, production and usage. We hope that the readers will find valuable information and exciting new material in its chapters.

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