RESEARCH PROJECT

Monitoring the microbial diversity and functional activities in response to land-use changes and deforestation under soybean and sugarcane cultivations

PFPMCG
Proc. FAPESP 2008/58114-3
June 2009 – May 2013
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<tr>
<th>RESEARCH TEAM</th>
<th>TITLE/FORMATION</th>
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<tr>
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<td>Molecular Biochemistry</td>
<td>Construction of Biochips</td>
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<td>Land-Use Changes</td>
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<td>Brigitte Feigl CNPq 2</td>
<td>Ph.D.</td>
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<td>Danielle G.G. Caldas CAPES-PNPD</td>
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<td>Microbiology</td>
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FAPESP Project – SAMPLING SITES
RESEARCH PROJECT – *Microbial Monitoring*
Linked to CAPES-Wageningen Project

1. Sinop – Mato Grosso State
2. Campo Verde – Mato Grosso State
3. EMBRAPA CPAP – Mato Grosso do Sul State
4. Sta. Rita do Passa Quatro – São Paulo State
5. Pradópolis – São Paulo State
ENVIRONMENTAL IMPACT OF AGRICULTURAL EXPANSION IN SOUTHWEST AMAZONIA

Rondônia and Mato Grosso = 1,200,000 km²
Forest
Dense Cerrado
Cerrado
Savanna

Slash
Burn
Pasture
Agriculture

In soil:
Organic matter dynamic
C and N stocks
Aggregation
Structure

Main culture
Soybean
Rice
Cotton
Maize

Succession
Fallow
Millet
Sorgum
Pasture/crop

Management
Conventional
Conservative

Social and economic consequences
Population
Income distribution
Education
Health
Sanitation

CO₂ equivalent
N₂O
CH₄

Modelling
Geographic Information System
Remote sensing
OBJECTIVES

1. Determine the microbial diversity and biochemical functioning associated to GHG (Greenhouse Gases) using mesocosms from soybean and sugarcane soils under contrasting land-use changes in the Amazonia.

2. Development of soil metagenomic studies and construct DNA chips ("ecochips") to target genes involved in the processes associated to GHG emission/sequestration, focusing mainly on the CO$_2$, CH$_4$, N$_2$O gases.
OBJECTIVES

3. Application of biochemical and molecular techniques including the ecochips for detection, quantification and correlation of the processes involved in the microbial C and N biogeochemical turnover.

4. Integration of bioinformatics and research data to apply in microbial genome and proteome studies for monitoring the microbes and their gene functioning under the soybean and sugarcane cultivations and determine the microbial role under conventional and sustainable agricultural systems.
PREVIOUS DATA FROM RESEARCH GROUP
Archaea were found in lower number in agricultural soils.
Forest = Acidobacteria and Verrucomicrobia were in high number but low in Cultivated Soil
Cultivated Soil = Firmicutes and Proteobacteria same shift pattern

SHIFTS FOUND
Ph.D. Project: “Soil microbial structure in different land uses on southwest Amazon and its relations with GHG emission”

**Doctorate Student: Daniel Renato Lammel**
Supervisor: Carlos Clemente Cerri
Co-supervisor: Siu Mui Tsai

Specific Objectives

- Analyze of functional genes related to GHG production/consumption: *mcrA, mmoX* and *norB*.
- T-RFLP, qPCR, sequencing
- Mesocosm and field studies

Associated to FAPESP project: “Impact of agriculture in Southwest Amazon”
Coordinator: Prof. Carlos Clemente Cerri
Land use change from Cerrado to Pasture and Soybean Field

Cerrado (Savanna) (Campo Verde – MT)

Pasture (Brachiaria brizantha)

Soybean Field
Land use change from Forest to Pasture and Soybean Field

Forest (Sinop)

Road between soybean Field and Pasture

Pasture (*Brachiaria brizantha*)

Soybean Field

Soil microbial structure in different land uses on southwest Amazon and its relations with greenhouse gases emission
“Molecular analysis of microbial communities in different land use on Southwest Amazon”

Land use change from Forest to Pasture and Soybean Field

**Mato Grosso State**

Forest – Deforested – 3rd Soybean – 7th Soybean – Pasture

**São Paulo State**

Forest – Sugarcane
Acidobacteria and Verrucomicrobia diversity in different land use in the agriculture expansion board on Southwest Amazon

MESOCOSMS

**Brachiaria**
Guanandi Farm
Deforested site - soil

**Soybean**
Jaguaruna Farm
1st harvest - soil

**Soybean**
Guanandi Farm
5th harvest - soil
Ph.D. Project: “*Acidobacteria* and *Verrucomicrobia* diversity in different land use in the agriculture expansion board on southwest Amazon”

**Ph.D. Student: Acácio Aparecido Navarrete**

Supervisor: Siu Mui Tsai

Co-Supervisor: Prof. J. van Veen (Dr. Eiko Kuramae)

**Specific Objectives**

- *Acidobacteria* and *Verrucomicrobia* diversity in soil and rizosphere – 16S rRNA gene clones library (primers phylum-specific) and pyrosequencing (bacterial universal primers);

- Hybridization potential in DNA microarrays - *Acidobacteria* and *Verrucomicrobia* 16S rRNA gene;

- Cultivation of *Acidobacteria* and *Verrucomicrobia*. 
Acidobacteria and Verrucomicrobia in bulk and soybean rhizosphere soils from Southeastern Brazilian Amazon arable fields.

A. Soybean root system (Glicine max Merril).
B. Brachiaria root system (Brachiaria brizantha L.).
A. Soil collection from soybean root.

SOIL RHIZOSPHERE SAMPLING
Acidobacteria
Quantitative Real Time PCR

There is a clear decrease of Acidobacteria from Forest to Soybean Cultivation (1th Cropping)

After continuous soybean cropping, Acidobacteria in soybean soils recover at comparable values in bulk soil but not completely recovered yet

1 = Primary Forest
2 = Soybean Crop
3 = Primary Forest
4 = Soybean Crop

2009
2010
There is a clear decrease of Verrucomicrobia from Forest to Soybean Cultivation (1th Cropping).

After continuous soybean cropping, Verrucomicrobia in soybean soils recover at comparable values.

1 = Primary Forest
2 = Soybean Crop
3 = Primary Forest
4 = Soybean Crop

2009

2010
Acidobacteria and Verrucomicrobia in bulk and soybean rhizosphere soils from Southeastern Brazilian Amazon arable fields (Study 1)  
(Acácio Navarrete)

- **Bulk soil adjacent forest**
  - Sampling
    - Two different sites
    - Oct 2009/Apr 2010

- **Bulk soil cropland**
  - Sampling
    - Two different sites
    - Oct 2009/Apr 2010

- **Soybean (Glicine max) rhizosphere**
  - Greenhouse exp.
  - Bulk soil from both arable fields

- **DNA isolation**
  - General bacteria PCR amplification (barcoded primers)
    - Pyrosequencing *

- **DNA isolation**
  - General bacteria PCR amplification (barcoded primers)
    - Pyrosequencing *

- **DNA isolation**
  - General bacteria PCR amplification (barcoded primers)
    - Pyrosequencing *

**Bacterial and phylum-specific (Acidobacteria and Verrucomicrobia) q-PCR**

- ANOVA (q-PCR assays)
  - Five replicate soil per site in each sampling period; duplicate greenhouse experiments per arable field soil; five replicate soil per greenhouse experiment.
  - Four independent q-PCR data sets for bulk soil adjacent forest, bulk soil cropland and soybean rhizosphere.

*Large multiplex amplicon pools for next-generation sequencing (GS FLX system 454 - Roche). Four independent samples collected from bulk soil adjacent forest, bulk soil cropland and soybean rhizosphere. Approximately 10,000 reads (350 pb) per sample. Estimative of variability (ANOVA) using relative abundance data.
Ph.D. Project: “Molecular analysis of microbial communities in different land use on Southwest Amazon”

**Ph.D. Student: Lucas William Mendes**

Supervisor: Siu Mui Tsai
Co-Supervisors: Prof. Wim van der Putten (Dr. Eiko Kuramae)
Prof. J. van Veen

**Specific Objectives**

- The rhizosphere effect
- T-RFLP Analysis of *Archaea* and *Bacteria* communities;
- Clone Library of functional genes related to GHG emissions;
- qPCR of functional genes related to GHG emissions.
Quantification of key genes steering the microbial nitrogen cycle in soils under different land-uses in Mato Grosso (Lucas Mendes)

Bulk Soil Adjacent Forest
Bulk Soil Deforested Site
Soybean crop field site

Bulk Soil Adjacent Forest
Bulk Soil Deforested Site
Pasture site

Brachiaria brizantha and Glycine max rhizosphere

DNA Isolation

Amplification of functional gene (nitrogen cycle)

qPCR*

*qPCR: Quantitative PCR or Real Time PCR, used to quantify, in number of copies, gene target from a microbial community.
*q-PCR enables detection and quantification of one or more specific sequences in a DNA sample.
Cultivating the “Uncultured” Bacteria
**CULTIVATION OF Acidobacteria and Verrucomicrobia UNDER CONTROLLED ATMOSPHERE**  
(Study 3)  
(Acacio Navarrete)

**TWO MEDIUM**  
- Medium Stevenson et al., 2004  
- Medium M13 (DSMZ) - *Verrucomicrobia*

**INCUBATION TIME**  
7, 14, 21 and 28 days

**ATMOSPHERE**  
2% O₂ (v/v), 5% CO₂ (v/v) and 93% N² (v/v)

**POSITIVE CONTROL PCR**  
*Acidobacterium capsulatum* (DSM 11244)  
*Verrucomicrobium spinosum* (DSM 4136)
UNDERGRADUATE RESEARCH

• Design and validation of primers for *Verrucomicrobia* 16S rRNA gene.
  ü Marcela Arnaldo

• Natural and induced re-establishment of degraded agricultural soils after soybean
  ü Marília Reichert, Caio Yoshiura

• Characterization of the land use systems in association to soil management - GIS
  ü Vanessa S. Rodrigues
**MOLECULAR TOOLS**

**FINGERPRINTING**
- T-RFLP
- DGGE
- ARISA

**SEQUENCING ANALYSES**
- SEQUENCING
  - 16S rRNA
  - Functional Genes (N and C Cycles)
- PYROSEQUENCING MICROARRAY
  - PRIMER DESIGN

**OTHER METHODS**

**CULTIVATION**
SIP = Stable Isotope Probing

**GAS CHROMATOGRAPHY**

**STUDY SITES:**
- SOYBEAN (MT, MS)
- SUGARCANE (SP)
- PASTURE (MT, MS)
- SOD-BASED/LIVESTOCK/ROWCROP INTEGRATION (MT, MS)