



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



RESEARCH TEAM	TITLE/FORMATION	ACTIVITY	INSTITUTION
Siu Mui Tsai CNPq 1A	Full Professor Microbiology and Microbial Ecology	Biodiversity Monitoring and SIP Metagenome	CENA/USP
Eliana G. M. Lemos CNPq 1B	Full Professor Molecular Biochemistry	Metagenome Construction of Biochips	FCAV/ UNESP
Carlos Clemente Cerri CNPq 1A	Full Professor Biogeochemistry Soil Chemistry	Land-Use Changes Greenhouse Gas Emission C sequestration	CENA/USP
Brigitte Feigl CNPq 2	Ph.D. Biogeochemistry Soil Biology	Land-Use Changes Greenhouse Gas Emission C sequestration	CENA/USP
Maria Victoria Ballester CNPq 2	Associate Professor Geoprocessing	Geoprocessing ArcGis	CENA/USP
Carlos Eduardo Cerri	Ph.D. Biogeochemistry	Organic Matter C sequestration	ESALQ/USP
Newton La Scala Jr.	Ph.D. Physics	Land-use changes Field Scale	FAC/UNESP
Danielle G.G. Caldas CAPES-PNPD	Ph.D. Plant Genetics	Bioinformatics	CENA/USP
Daniel Saito	Ph.D. Microbiology	Bioinformatics	FOP/UNICAMP UEA

FAPESP Project – SAMPLING SITES

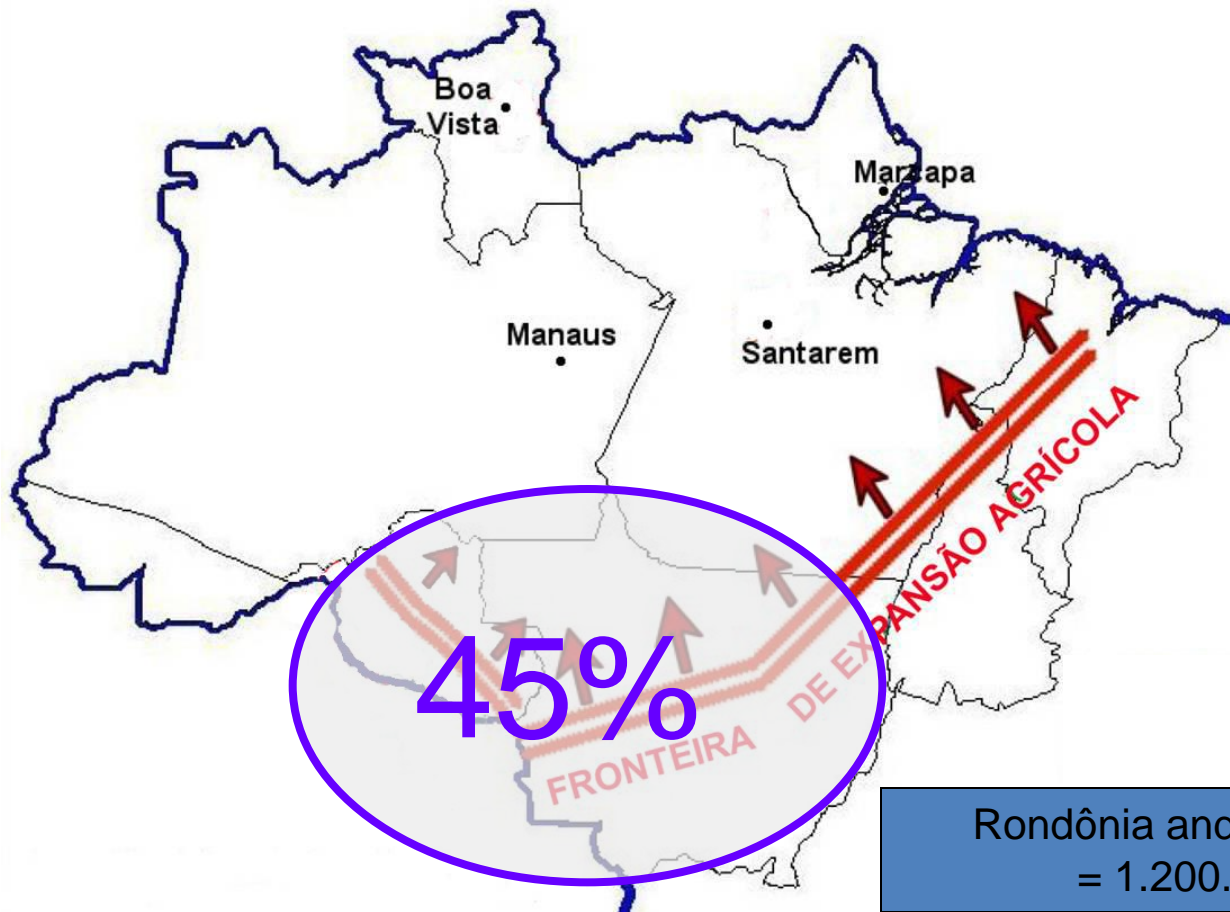
RESEARCH PROJECT – *Microbial Monitoring*

Linked to CAPES-Wageningen Project

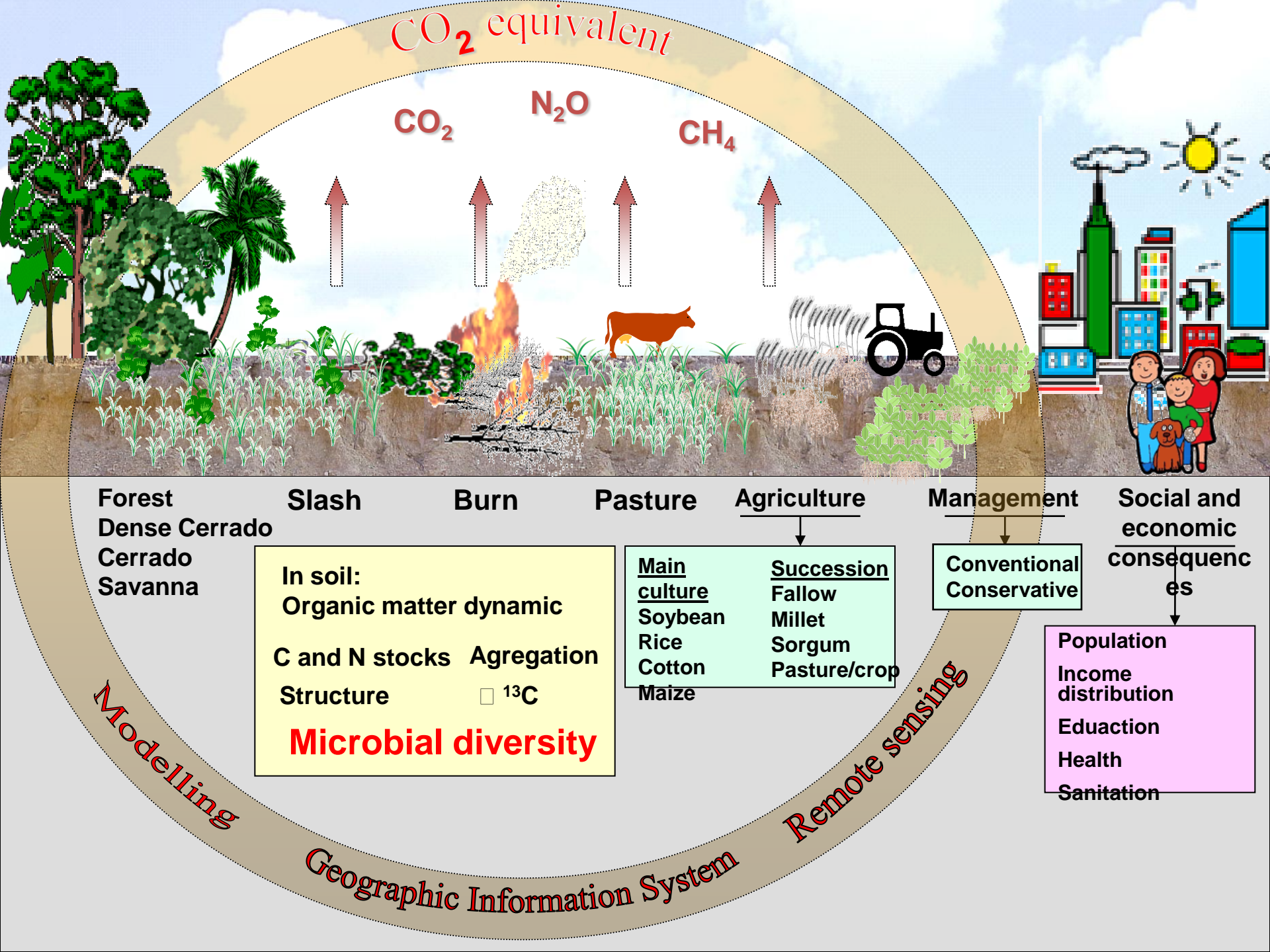


1. Sinop
– Mato Grosso State
2. Campo Verde
– Mato Grosso State
3. EMBRAPA CPAP
– Mato Grosso do Sul State
4. Sta. Rita do Passa Quatro
– São Paulo State
5. Pradópolis
– São Paulo State

ENVIRONMENTAL IMPACT OF AGRICULTURAL EXPANSION IN SOUTHWEST AMAZONIA



Rondônia and Mato Grosso
= 1.200.000 km²



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 CO₂, CH₄, N₂O gases.**

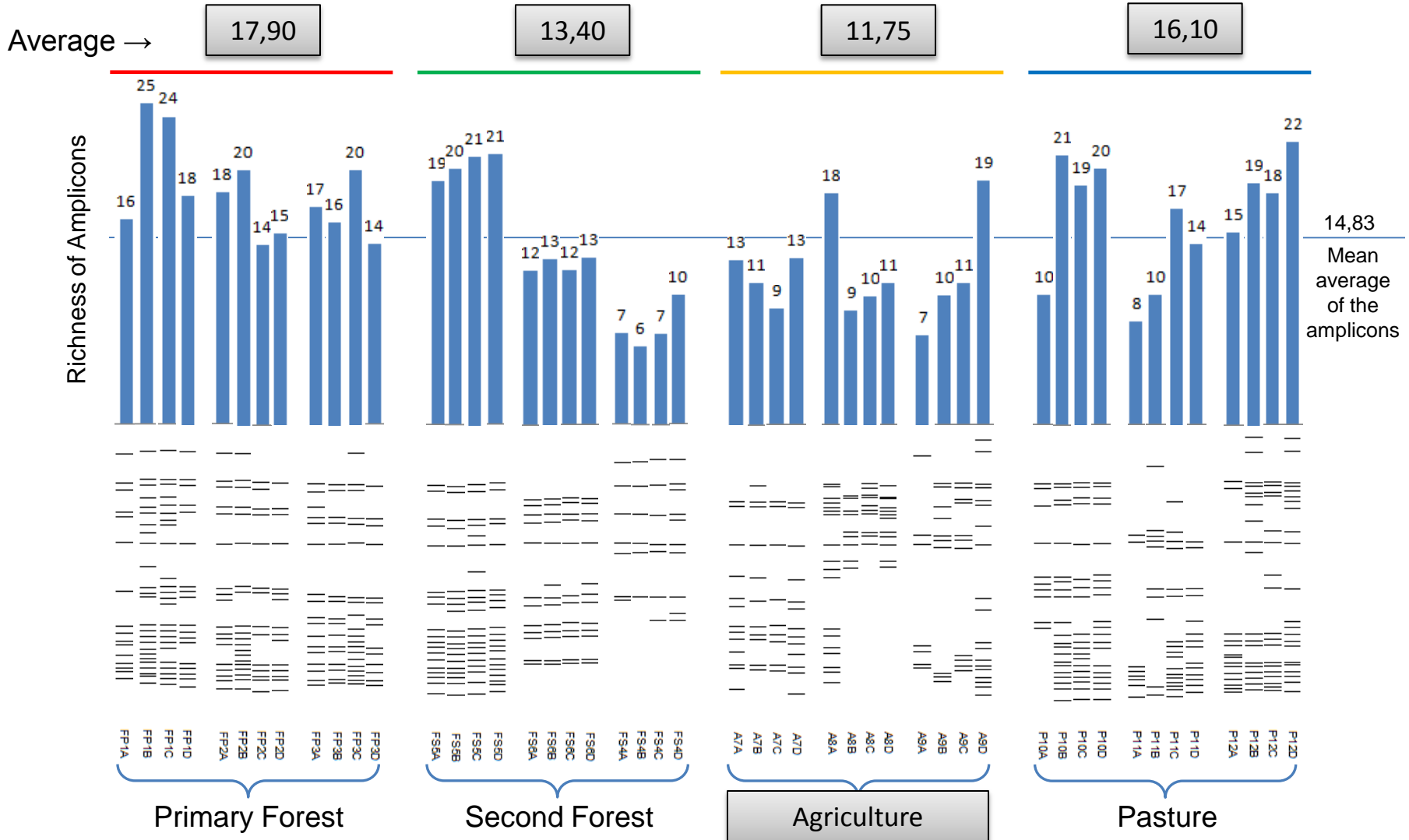
OBJECTIVES

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

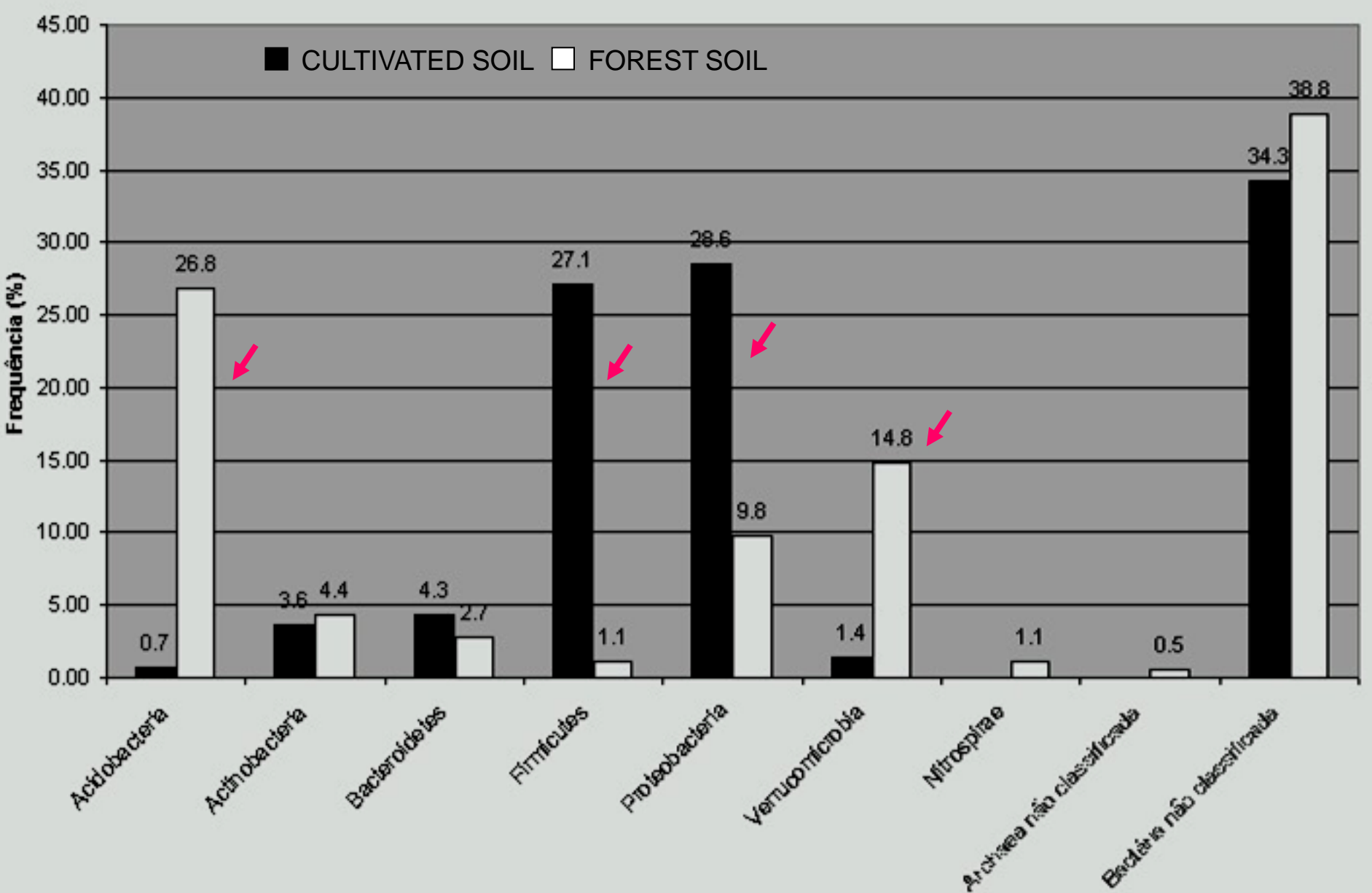
Archaea were found in lower number in agricultural soils

Western Amazonia - Benjamin Constant



SHIFTS FOUND

Forest = *Acidobacteria* and *Verrucomicrobia* were in high number but low in Cultivated Soil
Cultivated Soil = *Firmicutes* and *Proteobacteria* same shift pattern



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)

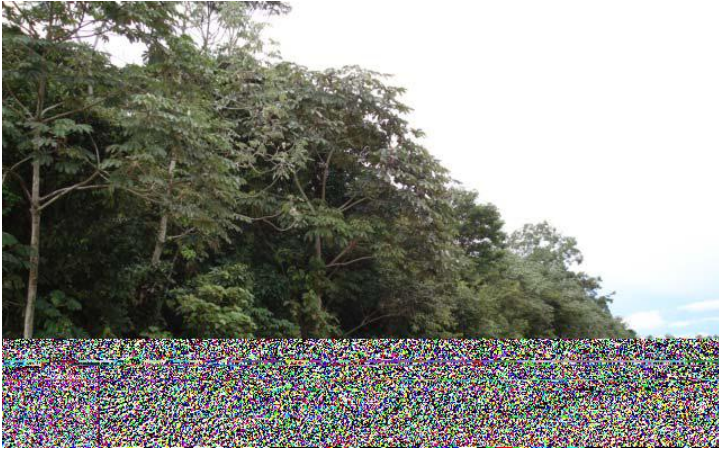


Pasture (*Brachiaria brizantha*)



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

Land use change from Forest to Pasture and Soybean Field

Mato Grosso State

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

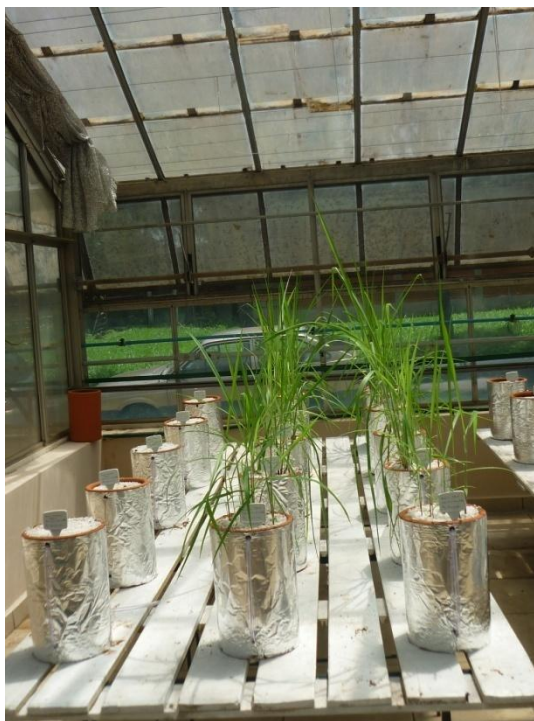
São Paulo State



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*.

Acácio A. Navarrete (PhD)
Siu Mui Tsai (supervisor CENA-USP).

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

A



B



C



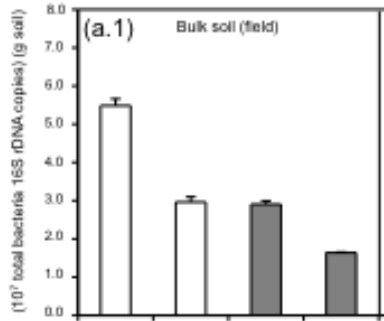
- A. Soybean root system (*Glicine max* Merrill).
- B. *Brachiaria* root system (*Brachiaria brizantha* L.).
- A. Soil collection from soybean root.

SOIL RHIZOSPHERE SAMPLING

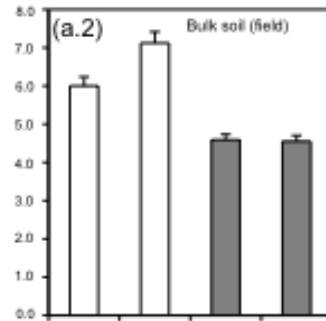
Acidobacteria

Quantitative Real Time PCR

Soybean Cropping
Year 1

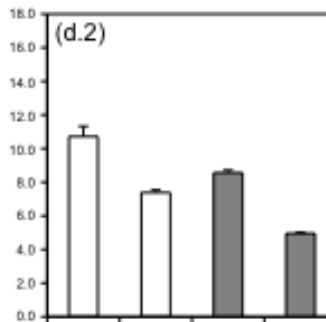
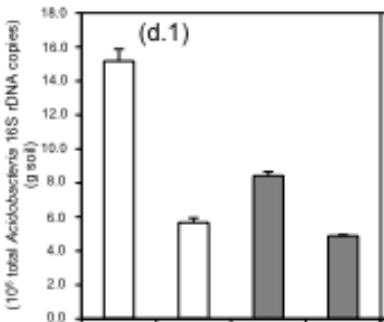
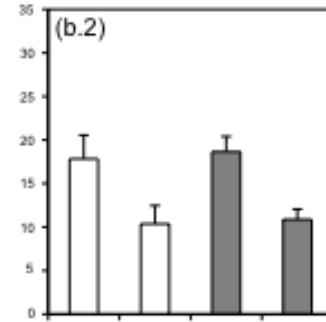
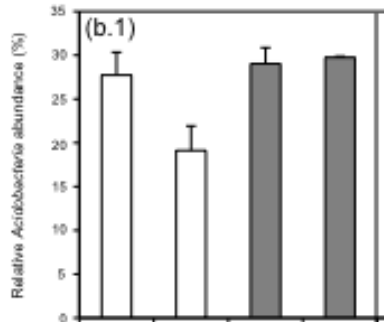


Soybean Cropping
Year 5



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



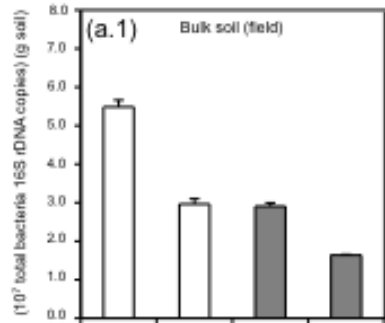
1 2 3 4

1 2 3 4

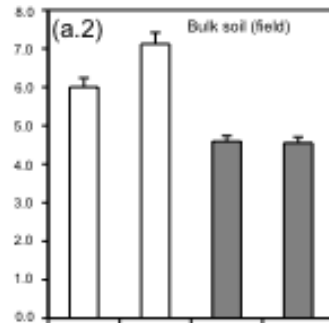
1 = Primary Forest	}	2009
2 = Soybean Crop		
3 = Primary Forest	}	2010
4 = Soybean Crop		

Verrucomicrobia Quantitative Real Time PCR

Soybean Cropping
Year 1

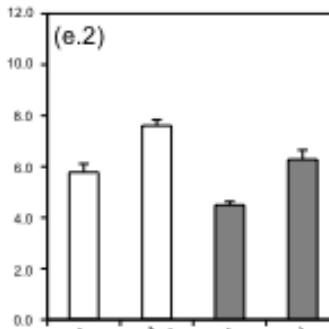
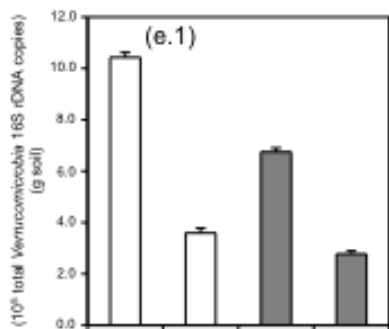
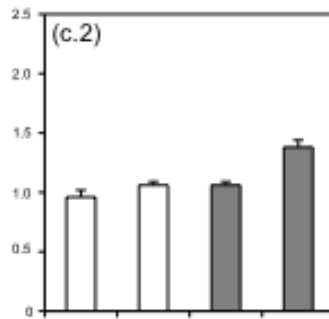
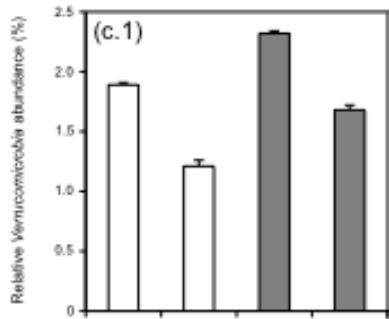


Soybean Cropping
Year 5



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

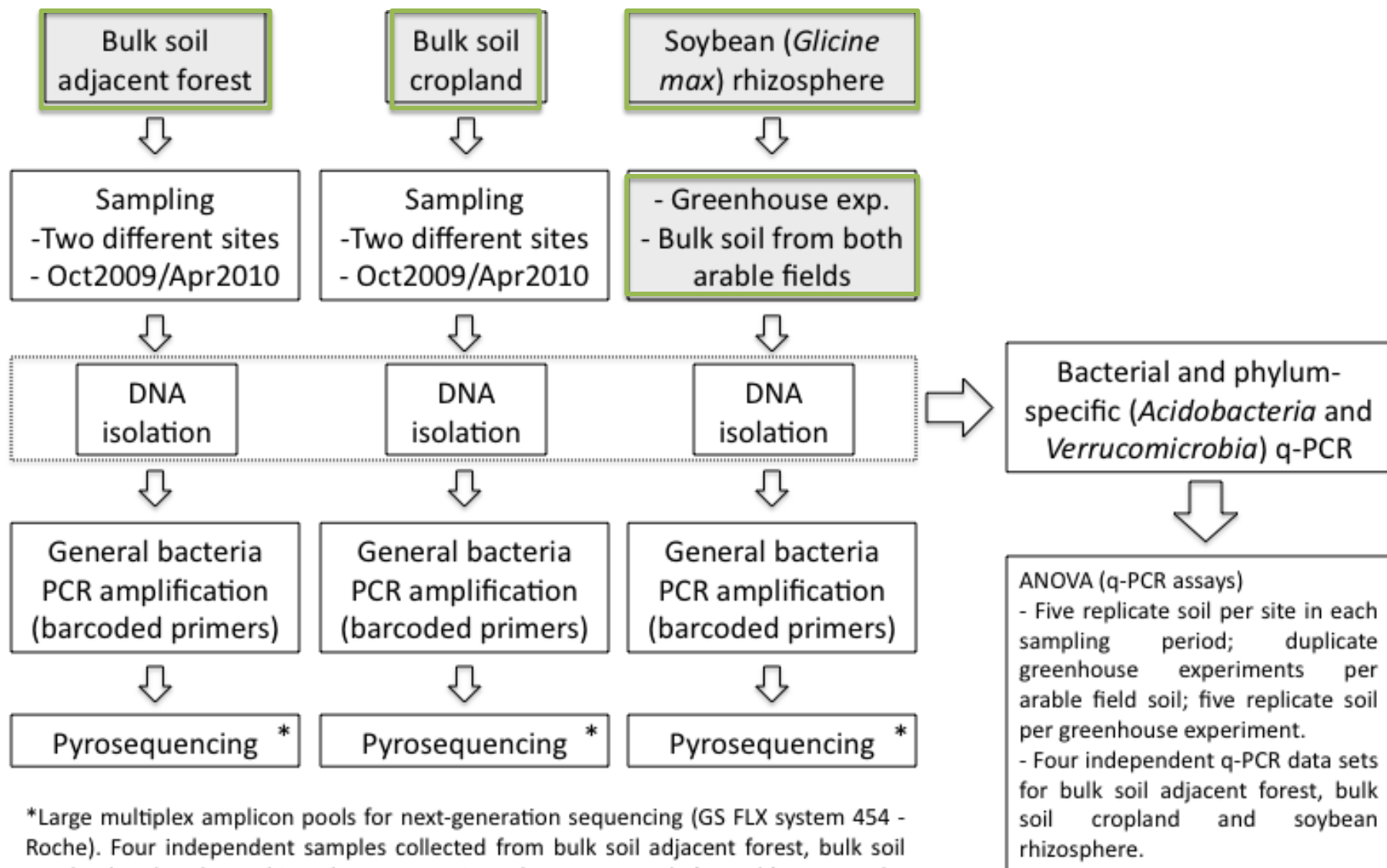


1 2 3 4

1 2 3 4

1 = Primary Forest	}	2009
2 = Soybean Crop		
3 = Primary Forest	}	2010
4 = Soybean Crop		

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



*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

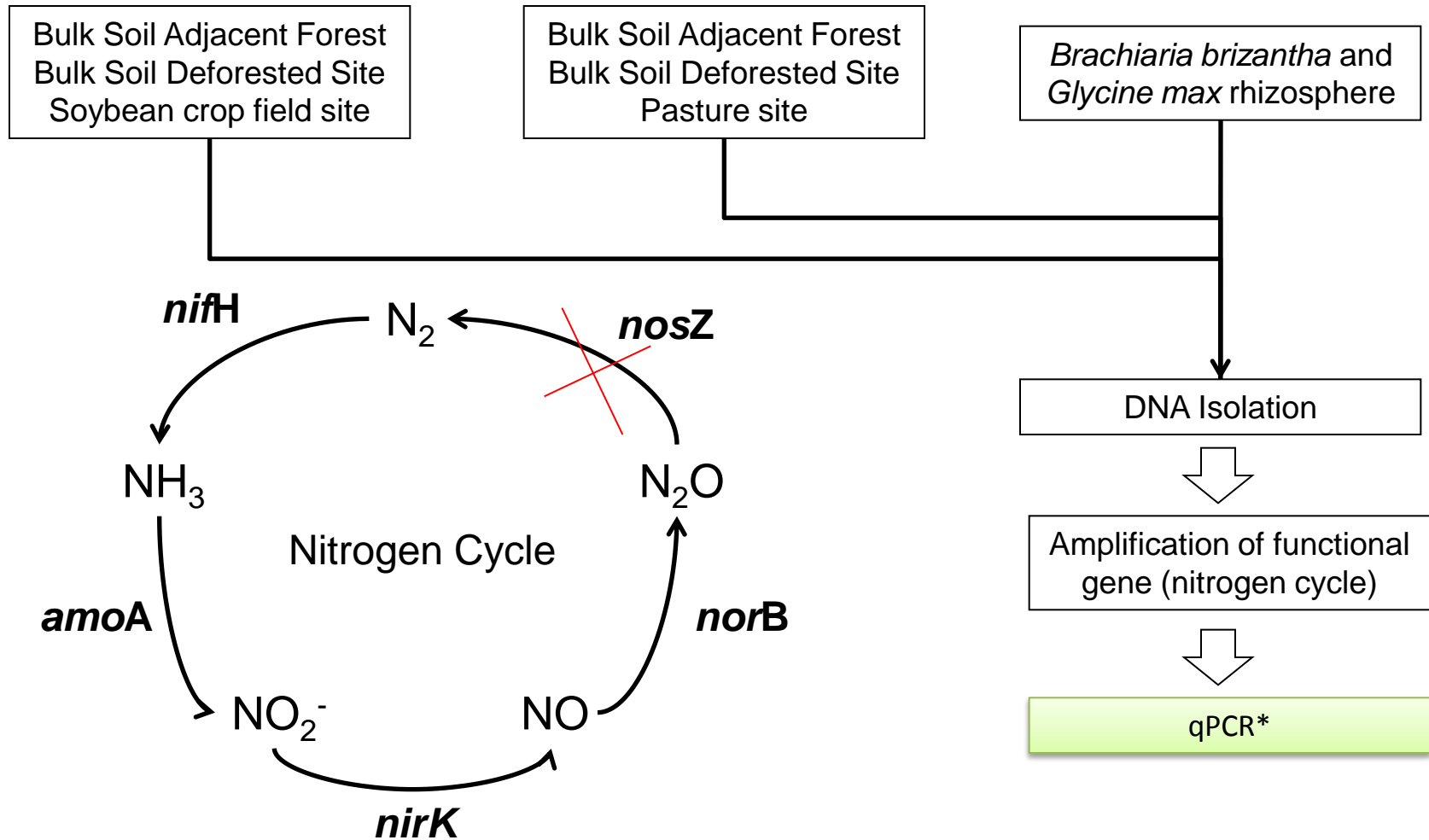
Co-Supervisors: Prof. Wim van der Putten (Dr. Eiko Kuramae)

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)



*qPCR: Quantitative PCR or Real Time PCR, used to quantify, in number of copies, gene target from a microbial community.

Detection key genes associated emissions of greenhouse gases under different land-uses in Brazilian Savanna (Clovis Borges)

Conventional Tillage
(Soybean)

No-tillage
(Soybean)

Integrated crop-livestock systems
(soybean/*Brachiaria Brizantha*)

Forest
(Semidecuiduos)

Soil Sampling Dec-2010 and Apr-2011

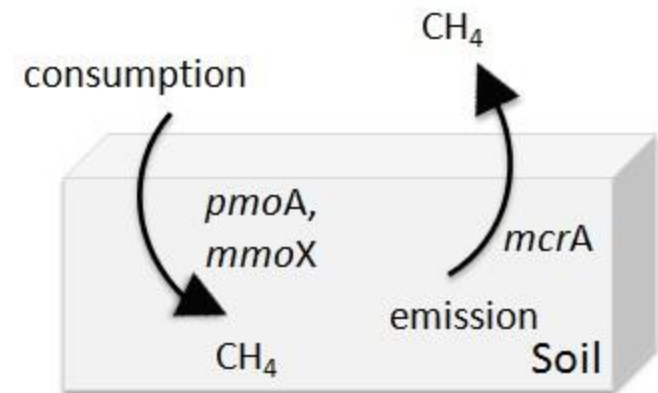
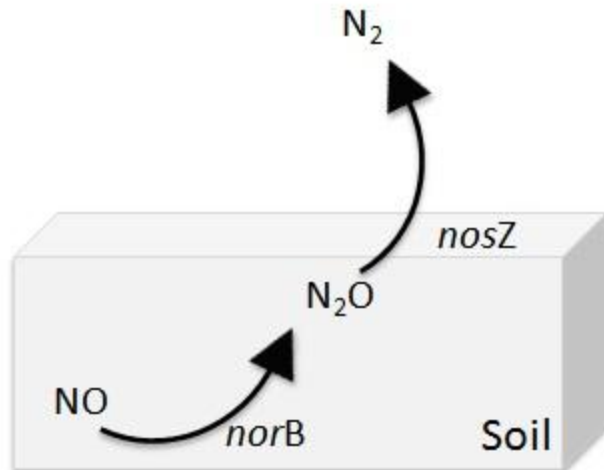
DNA isolation

Functional genes
(*pmoA*, *mcrA*, *nosZ*,
norB)

*q-PCR

Fluxes greenhouse
Gases (CO_2 , CH_4 , N_2O)

*q-PCR enables detection and quantification of one or more specific sequences in a DNA sample.

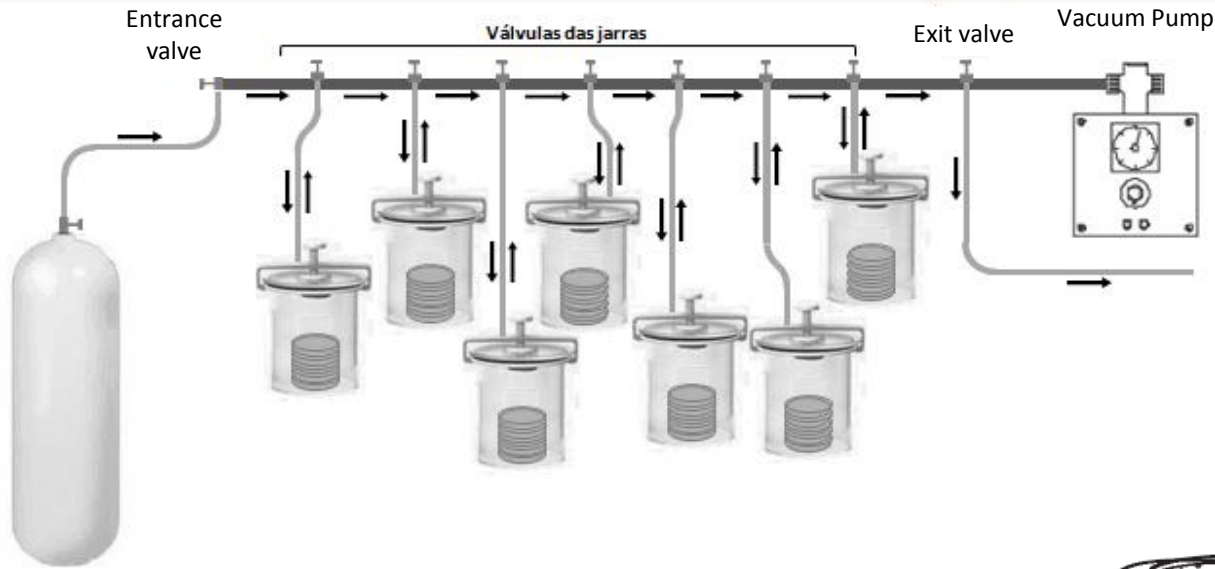


Cultivating the “Uncultured” Bacteria

CULTIVATION OF *Acidobacteria* and *Verrucomicrobia* UNDER CONTROLLED ATMOSPHERE

(Study 3)

(Acacio Navarrete)



Cylinder of gas

TWO MEDIUM

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

INCUBATION TIME

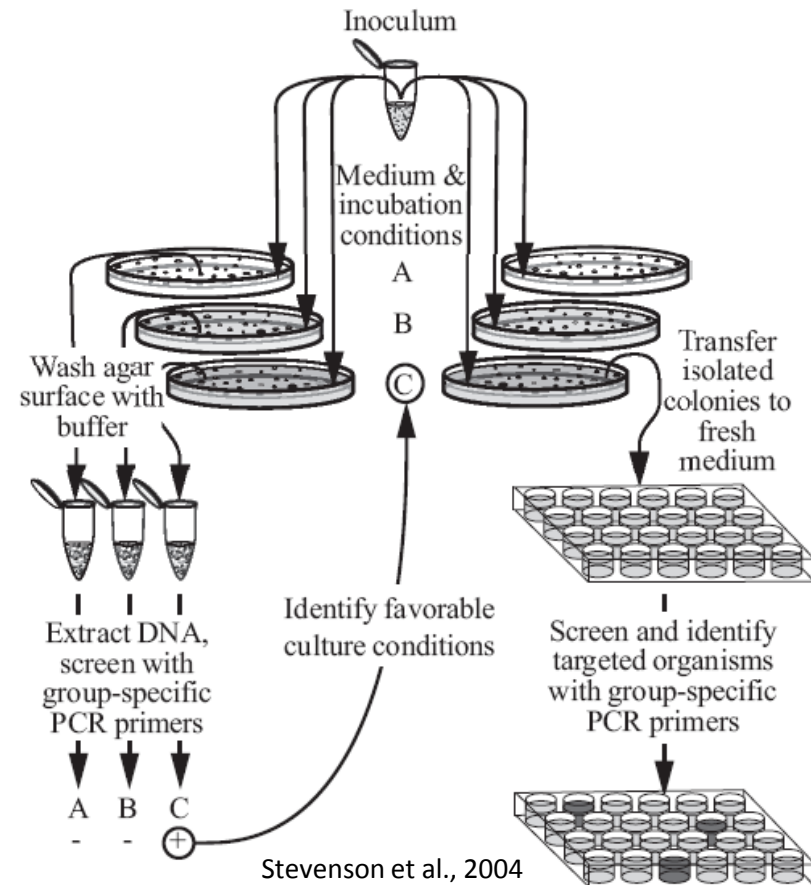
7, 14, 21 and 28 days

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)



Stevenson et al., 2004

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
 - ü 16S rRNA
 - ü 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)

