

# Uncovering a Hidden Methane Source: Grass Management and Soil Liming as Mitigation Strategies in Amazon Pasture Soils

Leandro Fonseca de Souza, Sc.D.

Assistant Professor

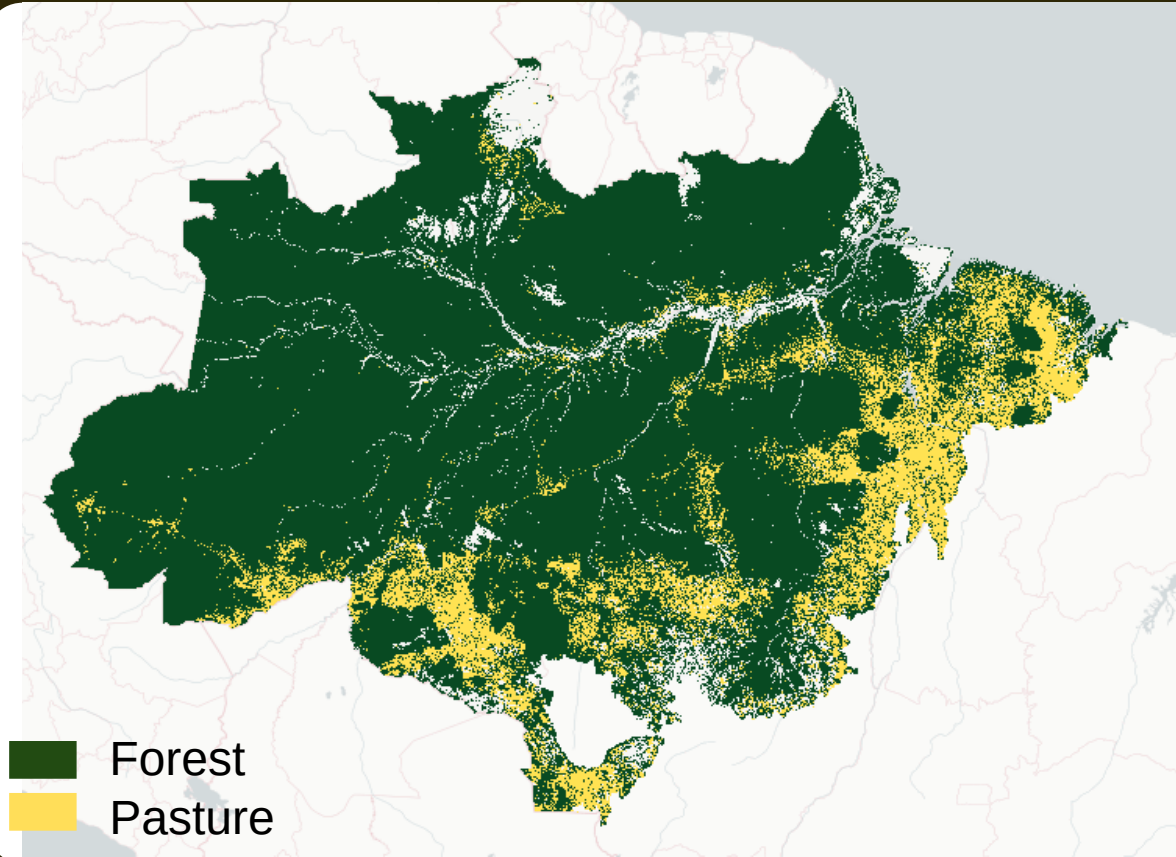
Federal University of Espirito Santo | UFES

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14<sup>th</sup> January 2026





Area:

**421 mi ha<sup>a</sup>**

Pastures:

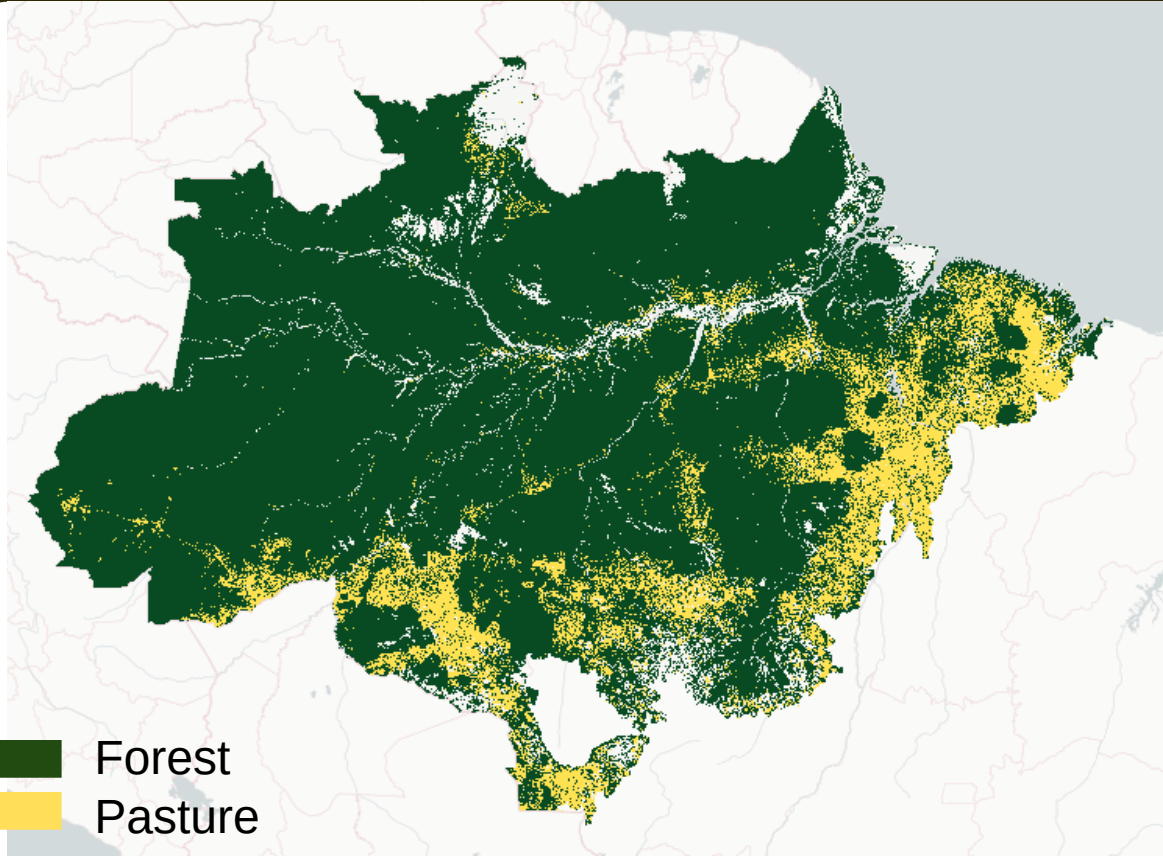
**56,1 mi ha<sup>a</sup>**

Degraded or under  
degradation pasture:

**> 30 mi ha<sup>a</sup>**

<sup>a</sup> MapBiomas – 2024

<https://brasil.mapbiomas.org/>



Area:

**421 mi ha<sup>a</sup>**

Pastures:

**56,1 mi ha<sup>a</sup>**

Degraded or under  
degradation pasture:

**> 30 mi ha<sup>a</sup>**



~24 mi ha

<sup>a</sup> MapBiomas – 2024

<https://brasil.mapbiomas.org/>



Ashes incorporation to  
the soil



pH



$\text{Al}^{3+}$

With time...



pH



$\text{Ca}^{2+}$

Without management...



Degradation

Need of liming to:

- Calcium and Magnesium supply
- Restoration of degraded areas



1976 – CH<sub>4</sub> is a greenhouse gas

JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 91, NO. D11, PAGES 11,791–11,802, OCTOBER 20, 1986

Emissions Of N<sub>2</sub>O, CH<sub>4</sub> and CO<sub>2</sub>  
From Tropical Forest Soils

MICHAEL KELLER

Department of Geology, Princeton University, Princeton, New Jersey

WARREN A. KAPLAN AND STEVEN C. WOFSY

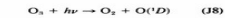
Center for Earth and Planetary Physics, Harvard University, Cambridge,  
Massachusetts

12 November 1976, Volume 194, Number 4266

SCIENCE

Greenhouse Effects due to Man-Made  
Perturbations of Trace GasesAnthropogenic gases may alter our climate by plugging  
an atmospheric window for escaping thermal radiation.

W. C. Wang, Y. L. Yung, A. A. Lacis, T. Mo, J. E. Hansen

tion with the hydroxyl radical OH (K2),  
with small additional contributions due  
to photolysis (J2) and reaction with  
O(<sup>1</sup>D) (K3). The all-important hydroxyl  
radical involved in reaction K2 is mainly  
derived fromThe concentration of OH is limited by  
reaction K2 and by reaction with carbon  
monoxide

## 1986 – Tropical forest soils sink atmospheric methane

## 1988 – Forest-to-pasture conversion can result in soil emissions

JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 101, NO. D13, PAGES 18,547–18,554, AUGUST 20, 1996

Consequence of forest-to-pasture conversion on CH<sub>4</sub> fluxes in  
the Brazilian Amazon BasinPaul A. Steudler,<sup>1</sup> Jerry M. Melillo,<sup>1</sup> Brigitte J. Feigl,<sup>2</sup> Christopher Neill,<sup>1</sup>  
Marisa C. Piccolo,<sup>2</sup> and Carlos C. Cerri<sup>2</sup>Tropical Deforestation: Some Effects on  
Atmospheric Chemistry

Report

By Thomas J. Goreau and William Z. de Mello

Ambio. 1988.

17(4): 275-281

## 1996 – Consistent measurements of pasture emissions

## 2000 – Importance of seasonality in soil CH<sub>4</sub> fluxes (east)



Seasonal variation of soil chemical properties and CO<sub>2</sub> and CH<sub>4</sub> fluxes in unfertilized and P-fertilized pastures in an Ultisol of the Brazilian Amazon

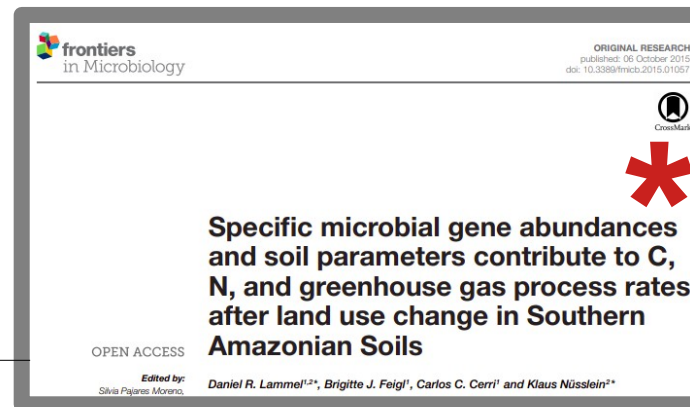
Silvana Aparecida Pavan Fernandes<sup>a,\*</sup>, Martial Bernoux<sup>b</sup>,  
Carlos C. Cerri<sup>a</sup>, Brigitte J. Feigl<sup>a</sup>, Marisa C. Piccolo<sup>a</sup>



## Land-Use Change and Biogeochemical Controls of Methane Fluxes in Soils of Eastern Amazonia

Louis V. Verchot,<sup>1,2\*</sup> Eric A. Davidson,<sup>1,2</sup> J. Henrique Cattaneo,<sup>2</sup>  
 and Ilse L. Ackerman<sup>1</sup>

## 2002 – Importance of seasonality in soil CH<sub>4</sub> fluxes (west)

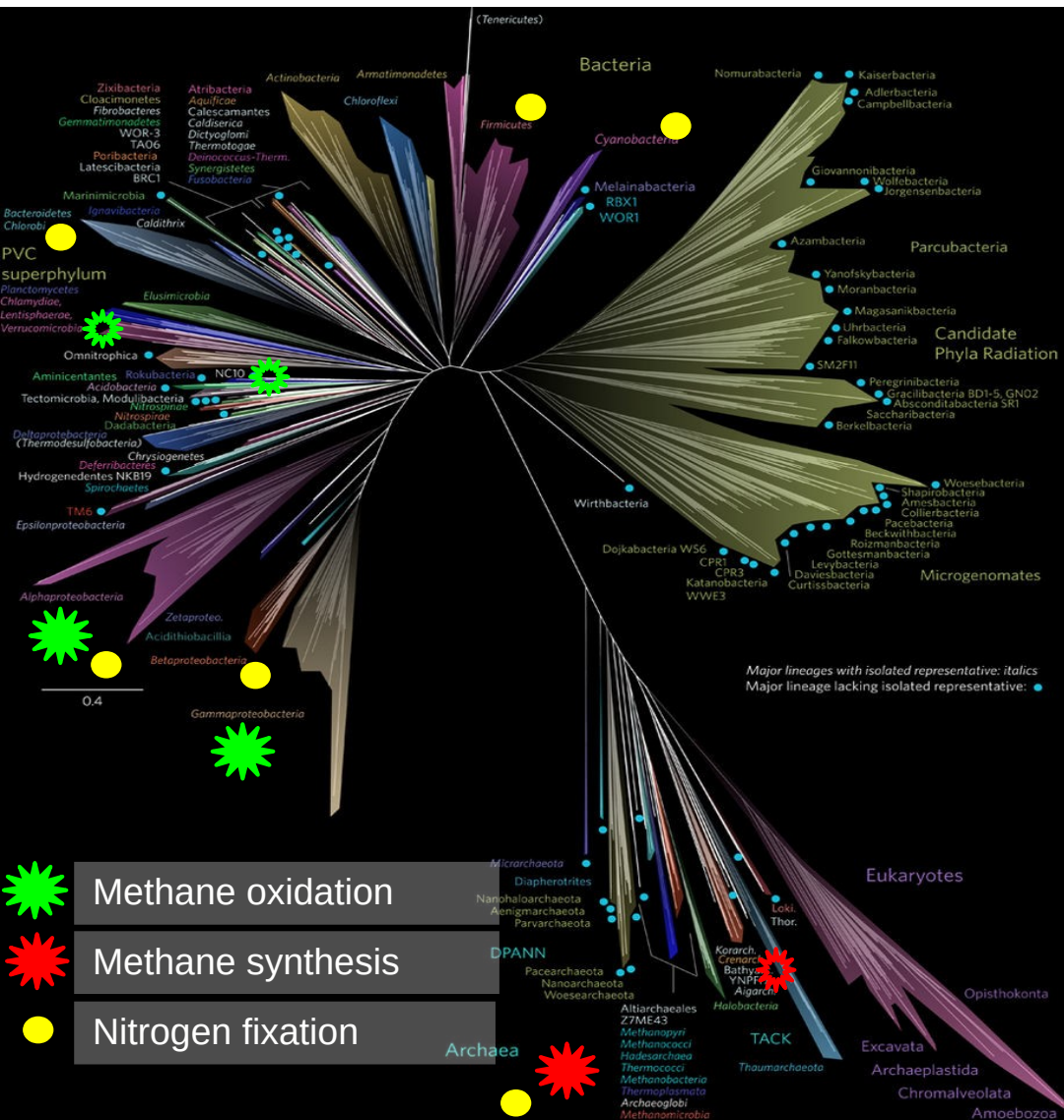


## Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in Southern Amazonian Soils

Daniel R. Lammel<sup>1,2\*</sup>, Brigitte J. Feigl<sup>1</sup>, Carlos C. Cerri<sup>1</sup> and Klaus Nüsslein<sup>2\*</sup>

## 2015 – First *insights* on Amazon soil methane microbiota





## Methane producers and consumers (best characterized)

### Methanogens

- ✓ Euryarchaeota
  - ✓ Methanomicrobiales
  - ✓ Methanocellales
  - ✓ Methanosarcinales
  - ✓ Methanobacteriales
  - ✓ Methanococcales
  - ✓ Methanopyrales
- ✓ Bathyarchaeota (2015)

### Methanotrophs

- ✓ Alphaproteobactérias
  - ✓ Methylocystaceae
  - ✓ Beijerinckiaceae
- ✓ Gamaproteobactérias
  - ✓ Methylococcaceae
  - ✓ Methylothermaceae
  - ✓ Crenotrichaceae
- ✓ Verrucomicrobia
  - ✓ Methylacidiphilaceae

## Growing evidence of multiple methane producers by secondary pathways

## Growing evidence of relevance of anaerobic methane oxidation

Methane oxidation

Methane synthesis

Nitrogen fixation

## Methane producers and consumers (best characterized)

### Methanogens

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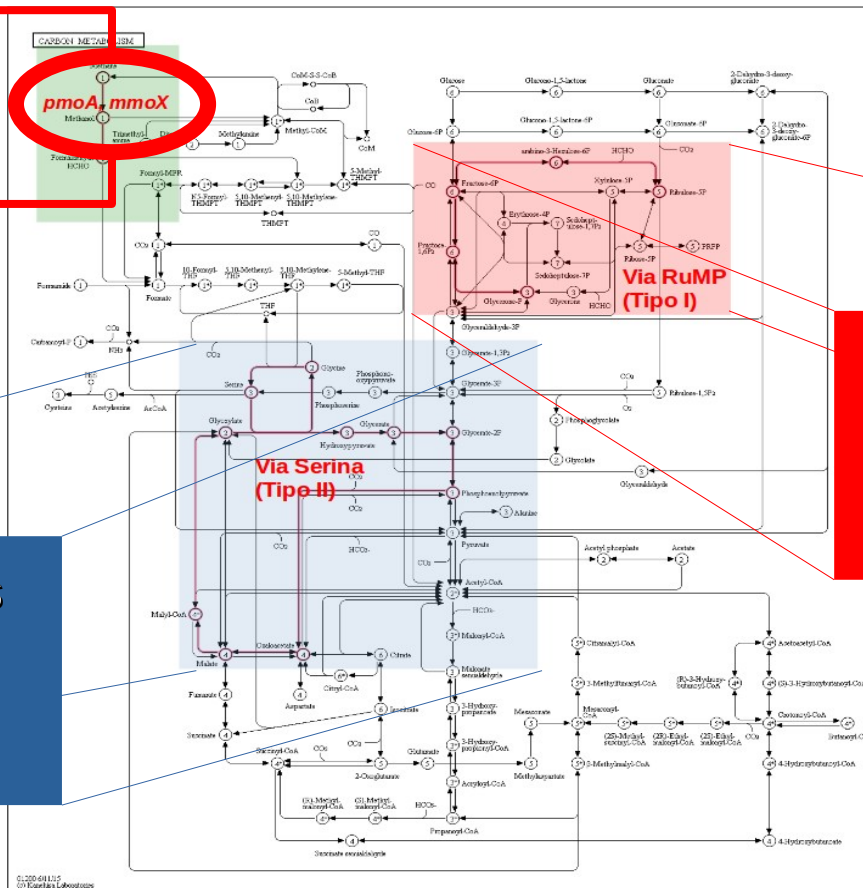


# Methane

# Methanol

## Type II Methanotrophs Alfaproteobacteria

## Serines pathway



## Type I Methanotrophs Gamaproteobacteria

## RUMP pathway



# How does grass coverage and soil liming influence methane fluxes in pastures?

Science of the Total Environment 838 (2022) 156225



Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: [www.elsevier.com/locate/scitotenv](http://www.elsevier.com/locate/scitotenv)



## Maintaining grass coverage increases methane uptake in Amazonian pastures, with a reduction of methanogenic archaea in the rhizosphere



Leandro Fonseca de Souza<sup>a,b,\*</sup>, Dasiel Obregon Alvarez<sup>a,c</sup>, Luiz A. Domeignoz-Horta<sup>b,d</sup>, Fabio Vitorino Gomes<sup>a</sup>, Cassio de Souza Almeida<sup>a</sup>, Luis Fernando Merloti<sup>a</sup>, Lucas William Mendes<sup>a</sup>, Fernando Dini Andreote<sup>e</sup>, Brendan J.M. Bohannan<sup>f</sup>, Jorge L. Mazza Rodrigues<sup>g</sup>, Klaus Nüsslein<sup>b</sup>, Siu Mui Tsai<sup>a</sup>

<sup>a</sup> Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil

<sup>b</sup> Department of Microbiology, University of Massachusetts, Amherst, MA, USA

<sup>c</sup> Applied Soil Ecology Lab, School of Environmental Sciences, University of Guelph, Guelph, ON, Canada

<sup>d</sup> Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

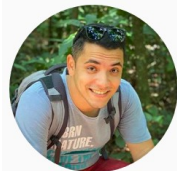
<sup>e</sup> Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil

<sup>f</sup> Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

<sup>g</sup> Department of Land, Air and Water Resources, University of California Davis, CA, USA

## Experiment 1

Amazon West  
~250 dias



## Methodology - Study 1

Sequencing 16S *rRNA*

qPCR – *pmoA* and *mcrA*

CH<sub>4</sub> fluxes

## Field Study

Belterra/PA

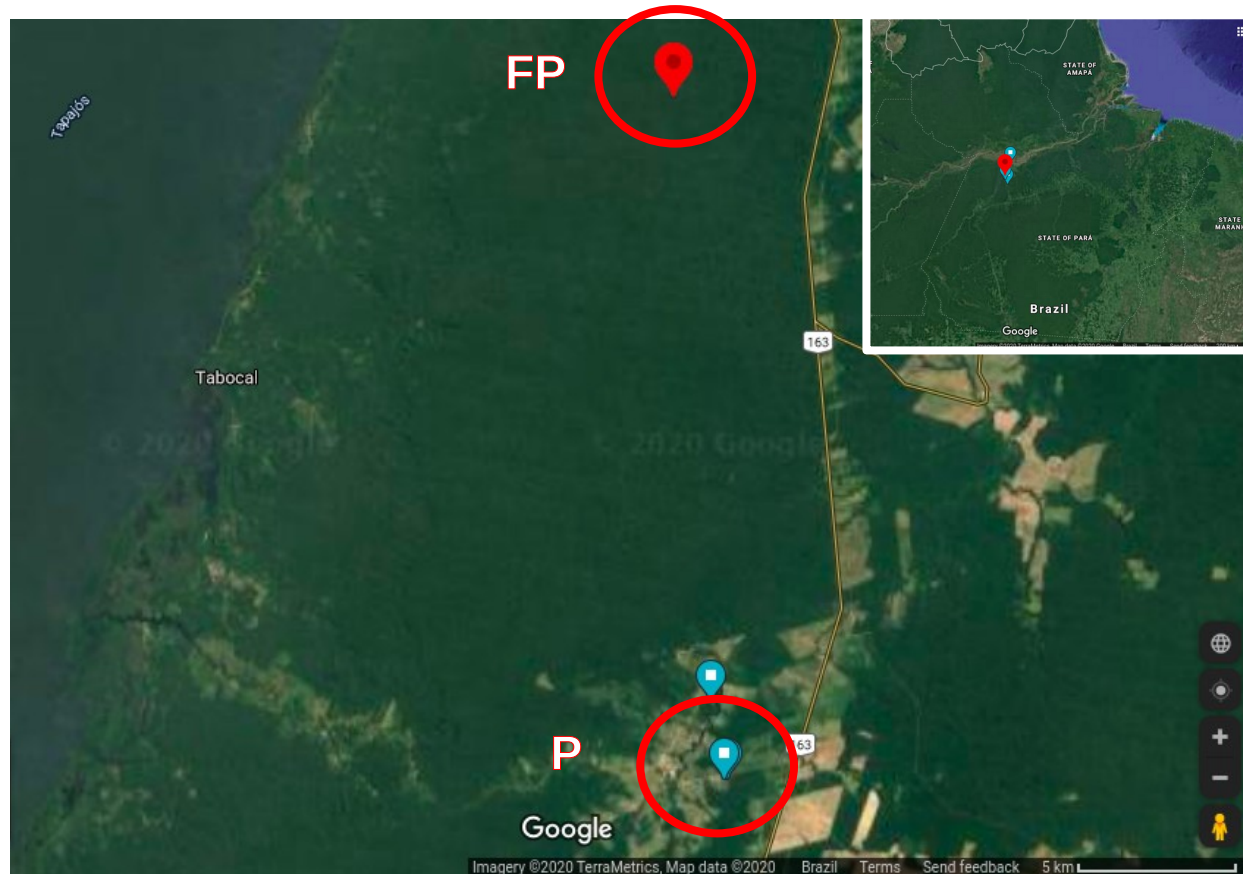






## Ariquemes/RO Amazon West

- Pastureland – 1972
- Fazenda Nova Vida
- Forest fragment
- Smallholding
- Soil type: Yellow–red Oxisols
- Texture: medium clay to sandy
- Sampling (0–10 cm): Apr/2017 (rainy season)
- Transect sampling: 5 points, 50 m apart



## Belterra-Santarém/PA Amazon East

- Pastureland – 1989–1994
- Smallholding
- National forest
- Conservation area
- Texture: medium clay to sandy
- Sampling (0–10 cm): Aug/2019 (end of the rainy season)
- Transect sampling: 5 points, 50 m apart

## Tapajós National Forest

Pasture 1



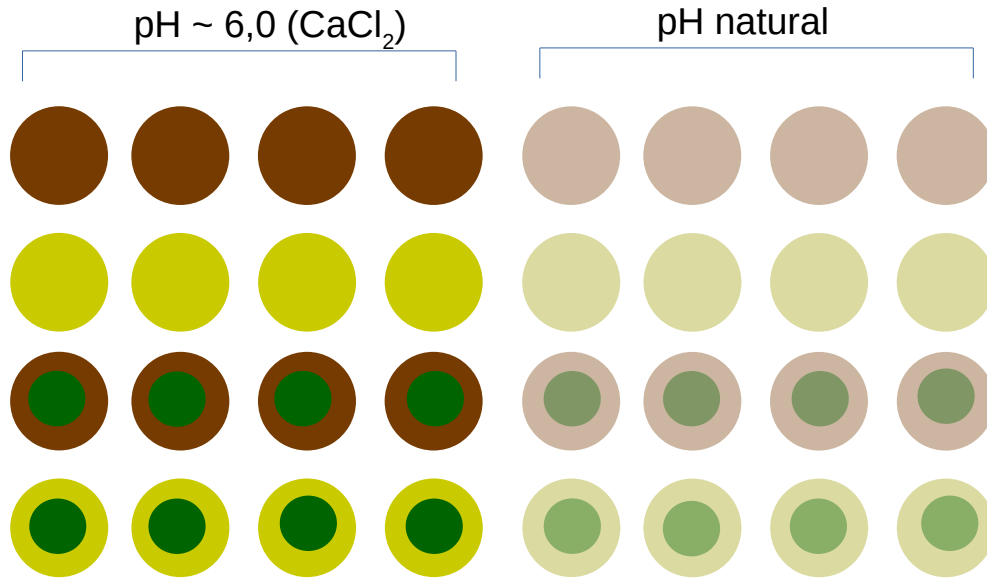
Pasture 2



## Belterra-Santarém/PA Amazon East

- Pastureland – 1989–1994
- Smallholdings
- Texture: sandy
- Sampling period: Aug/2019 (rainy season)
- Sampling design: 5 points forming a quadrilateral plus a central point; 100 m side length
- Pasture condition: few signs of degradation
- Samples: bulk soil, rhizosphere, and adjacent soil





- 10 L clay pots
- 5 kg of soil sieved to 5 mm
- 10 cm soil layer
- Moisture: 70% of water-holding capacity
- Adjustment: every 2–4 days

- ✓ CH<sub>4</sub>, CO<sub>2</sub>, and water vapour
- ✓ No daily calibration required
- ✓ Cylindrical chambers: 12 cm radius × 30 cm height; volume ~6 L
- ✓ Measurement time: 10 min; one data point every 10 s
- ✓ Linear fitting model to estimate the rate of change
- ✓ Measurement times (days):
  - ✓ Exp 1: 7, 18, 28, 84, 96, and 108
  - ✓ Exp 2: 7, 13, 21, 28, and 35

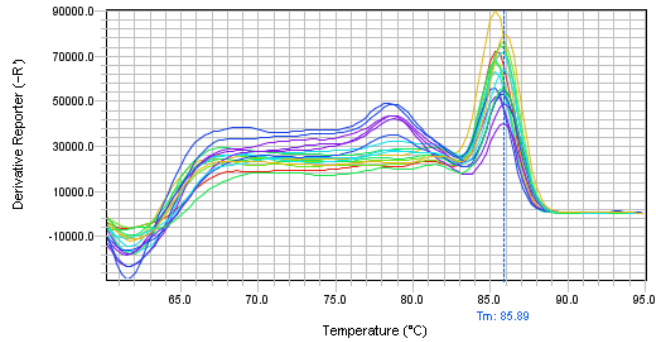


Laser spectroscopic analyser (ABB, Switzerland)



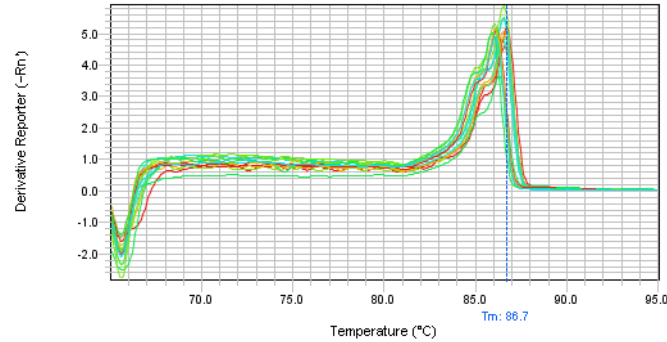
*mcrA*

Melt Curve



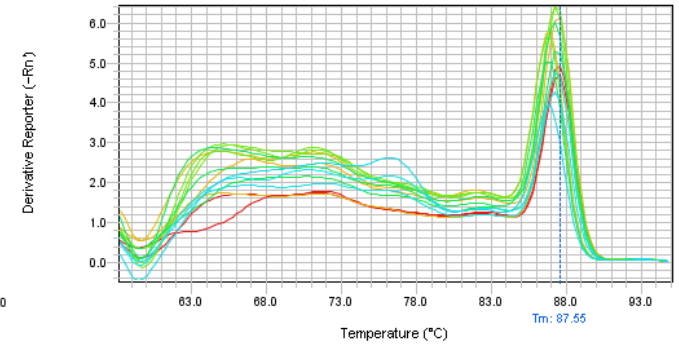
*mmoX*

Melt Curve

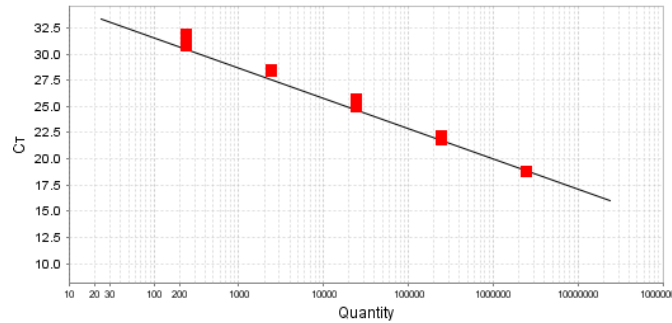


*pmoA*

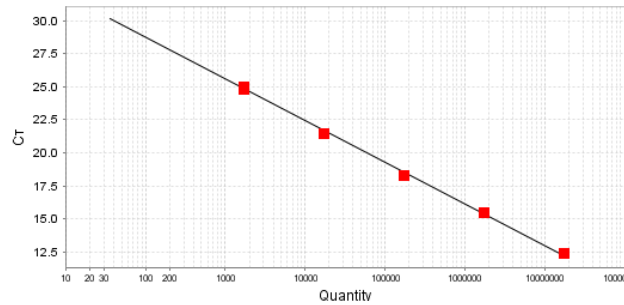
Melt Curve



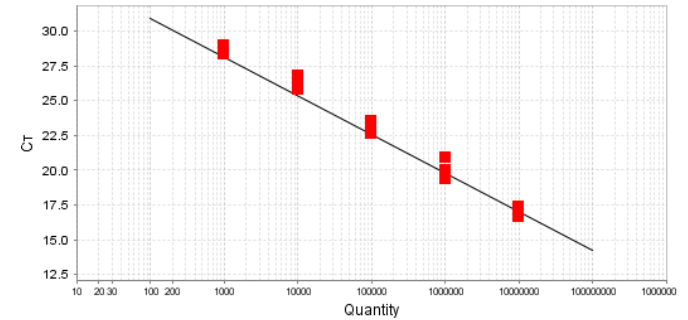
Standard Curve



Standard Curve



Standard Curve



\* linreg



## Sequencing

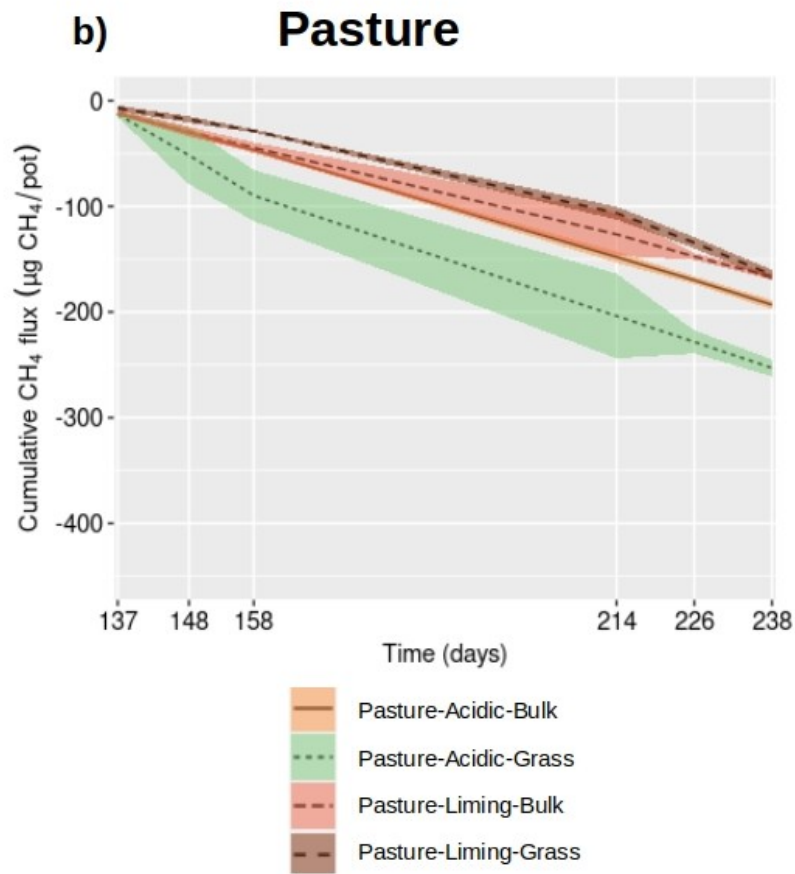
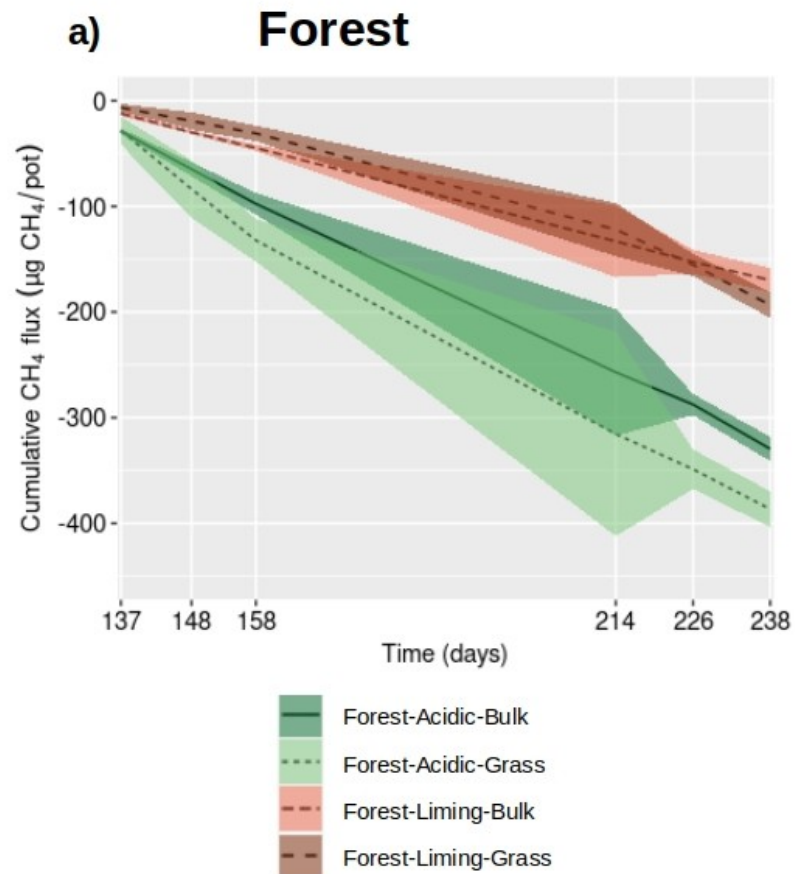
- University of Sao Paulo
- Illumina MiSeq v3 (2x250pb)
- *Paired-end*
- 16S *rRNA* (v4)
- 515F (Parada)/ 806R (Apprill)

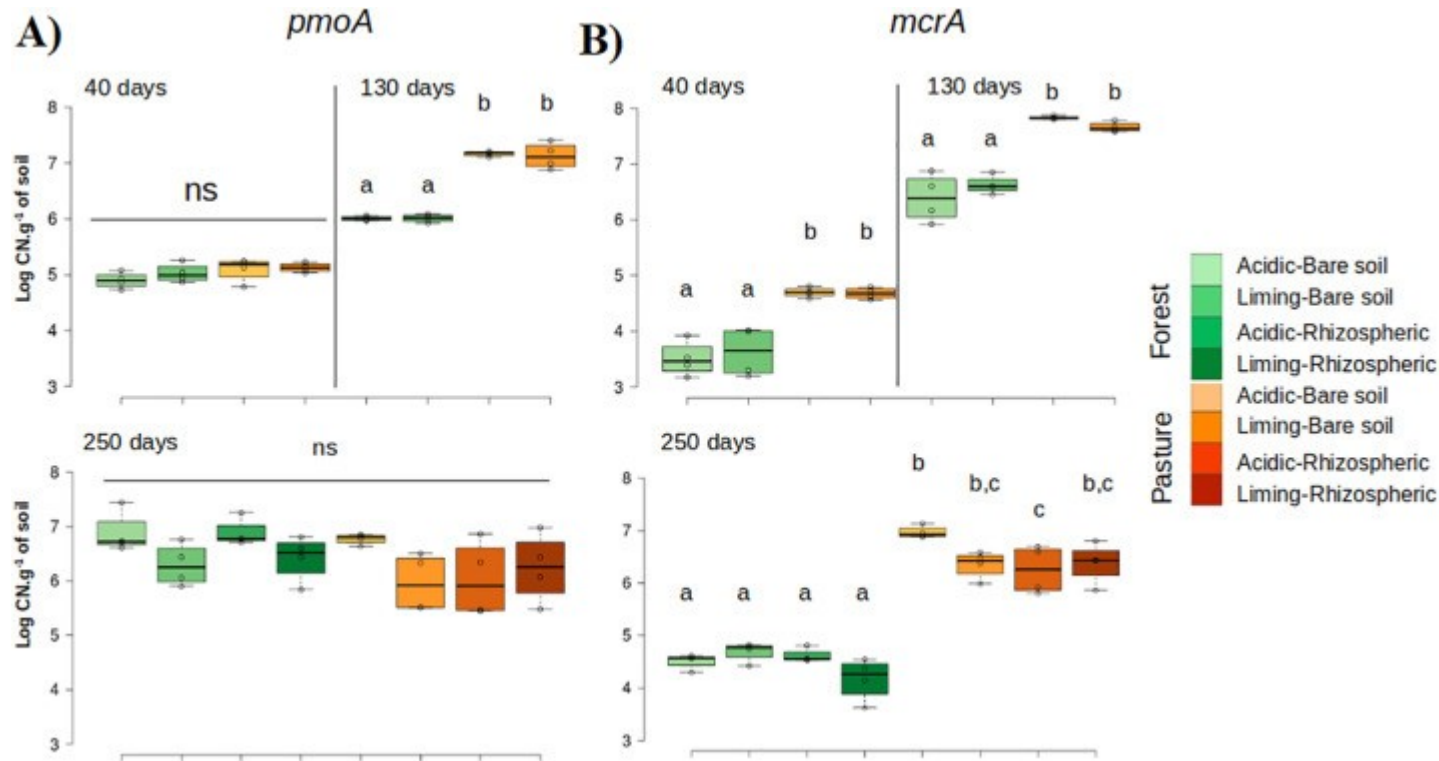


## Data processing

- Qiime2
- Dada2 (ASVs)
- SILVA 132
- RDA/ PCA
- PERMANOVA
- DEICODE/ QURRO – differential abundance of taxa
- ANOVA and Tukey HSD







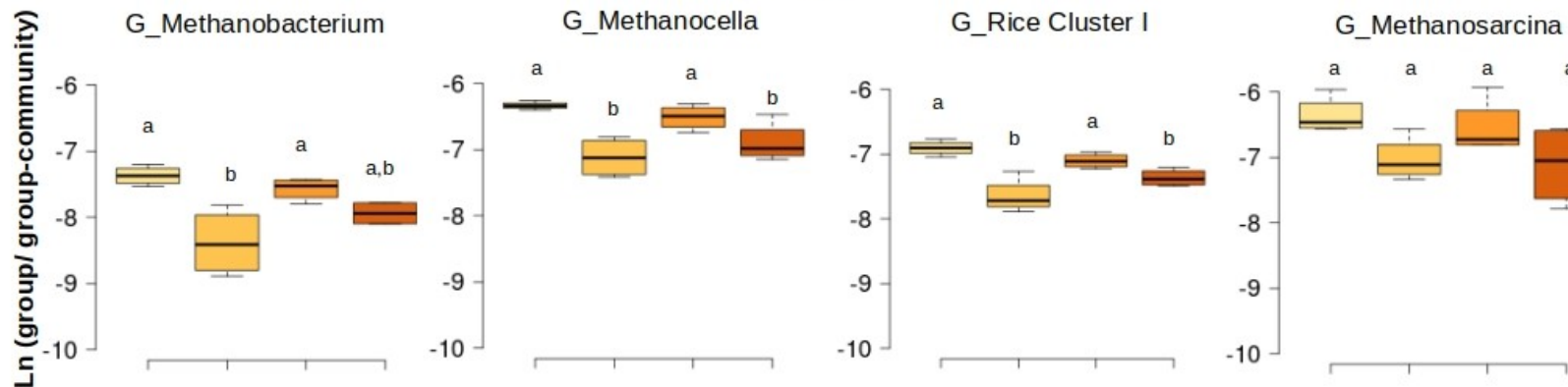
- *mcrA* is more abundant in pasture soils
- *pmoA* tends to decrease in forest soils under liming (ns)
- *mcrA* is significantly reduced in the rhizosphere of pasture soils
- *mmoX* shows no significant changes

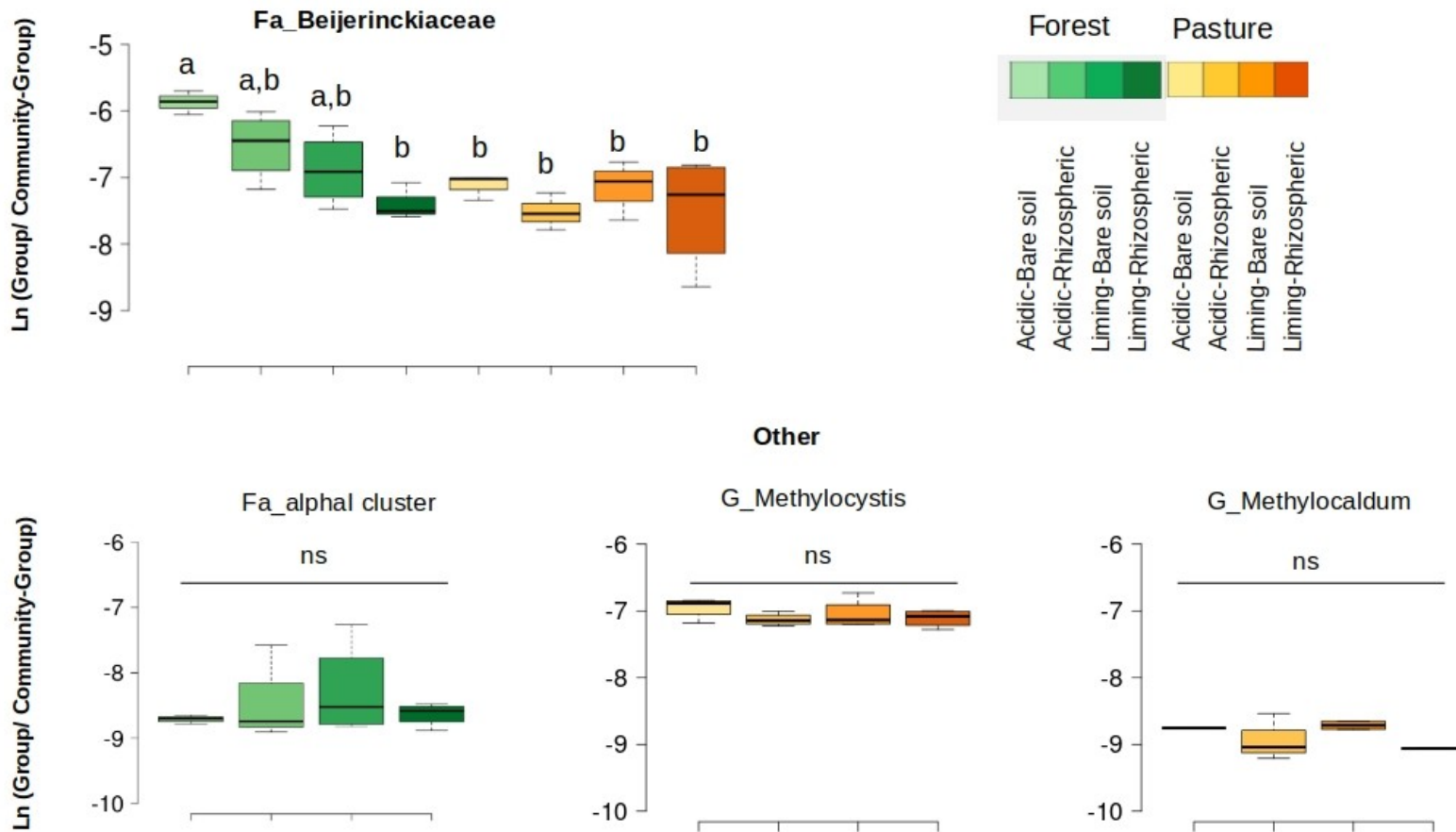


## Forest



## Pasture





- Grass coverage increases methane uptake in pasture soils compared to bare soils.
- Methanogens were reduced by 10 fold in the grass rhizosphere compared to bulk soil.
- Soil liming can compromise the capacity of forest and pasture soils to sink methane.
- Pasture management strategies have potential to mitigate soil methane emissions.



Methanogens are reduced in pasture grass rhizosphere, and explain at least part of the changes in  $\text{CH}_4$  fluxes.

What about the forest soils? They change in flux with liming, but not much the abundance of microbes.

Maybe the activity?

*Journal of Applied Microbiology*, 2025, 136(1), lxae303

<https://doi.org/10.1093/jambio/lxae303>

Advance access publication date: 19 December 2024

Research Article

JOURNAL OF  
APPLIED  
MICROBIOLOGY

OXFORD  
UNIVERSITY PRESS

## Soil pH modulates the activity of low-affinity methane oxidation in soils from the Amazon region

Leandro Fonseca de Souza<sup>1,2,3,\*</sup>, Fernanda Mancini Nakamura<sup>1,4</sup>, Marie Kroeger<sup>5</sup>,  
Dasiel Obregon<sup>1,6</sup>, Moacir Tuzzin de Moraes<sup>1,7</sup>, Mariana Gomes Vicente<sup>1</sup>,  
Marcelo Zacharias Moreira<sup>1</sup>, Vivian Helena Pellizari<sup>4</sup>, Siu Mui Tsai<sup>1</sup>, Klaus Nüsslein<sup>3</sup>

<sup>1</sup>Center for Nuclear Energy in Agriculture, University of Sao Paulo, Piracicaba, SP 13400-970, Brazil

<sup>2</sup>Center for Agricultural Sciences and Engineering, Federal University of Espirito Santo, Alegre, ES 29500-000, Brazil

<sup>3</sup>Department of Microbiology, University of Massachusetts, Amherst, MA 01003-9298, USA

<sup>4</sup>Oceanographic Institute, University of Sao Paulo, Sao Paulo, SP 05508-120, Brazil

<sup>5</sup>Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545-1663, USA

<sup>6</sup>School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada

<sup>7</sup>Department of Soil Science, Luiz de Queiroz College of Agriculture, University of Sao Paulo, Piracicaba, SP 13418-900, Brazil

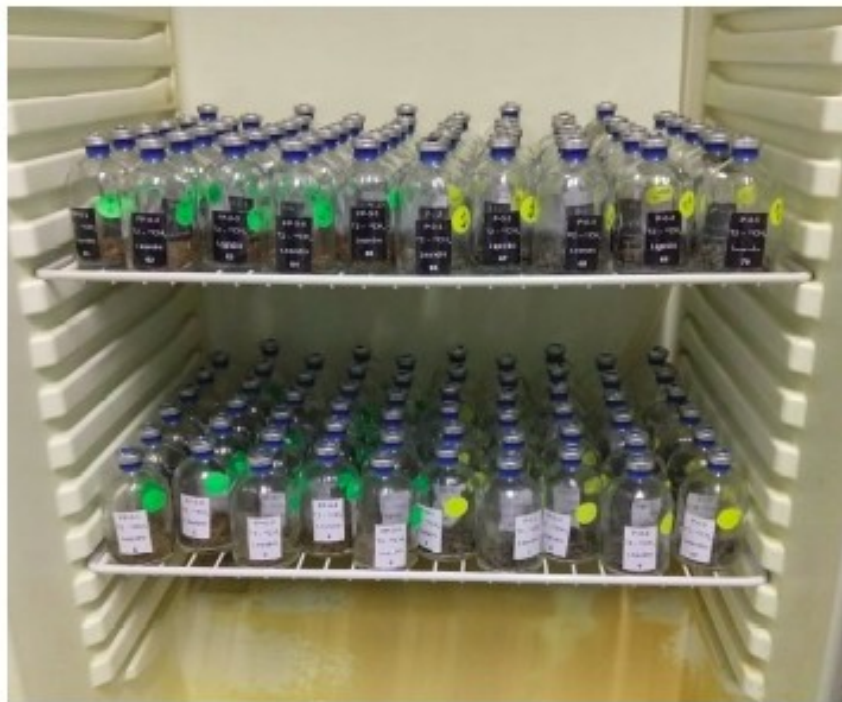
\*Corresponding author. Center for Nuclear Energy in Agriculture, University of Sao Paulo, Piracicaba, SP 13400-970, Brazil.

E-mail: [leandro\\_fonseca@alumni.usp.br](mailto:leandro_fonseca@alumni.usp.br)



## Experiment design

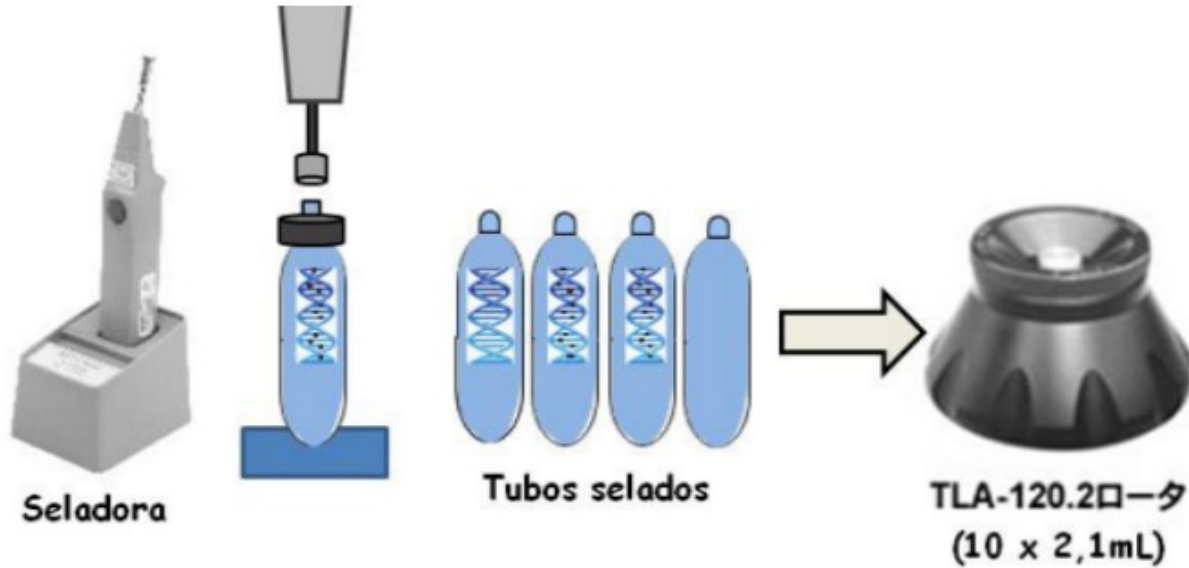
- Microcosms
  - Hermetically sealed 1.65 L jars (5 replicates)
  - 400 g of soil
- Temperature: 25 °C (BOD)
- Moisture: 70–80% of water-holding capacity
- $^{12}\text{CH}_4$  concentrations:
  - ~2 ppm
  - ~200 ppm
  - ~2,000 ppm
  - ~20,000 ppm (forest soils only)
- Duration: 24 days
- $\text{CH}_4$  flux measurements: weekly



## Experiment

- ✓ Microcosms sealed with rubber lids
  - 120 mL
  - 10 g of soils
  - Temperature: 25 °C BOD
- ✓ Moisture: 70-80%
- ✓ Concentration of  $^{13}\text{CH}_4$ 
  - ~10,000 ppm
- ✓ 24 days
- ✓ Readings and replacement of  $^{13}\text{CH}_4$  every 2-4 days

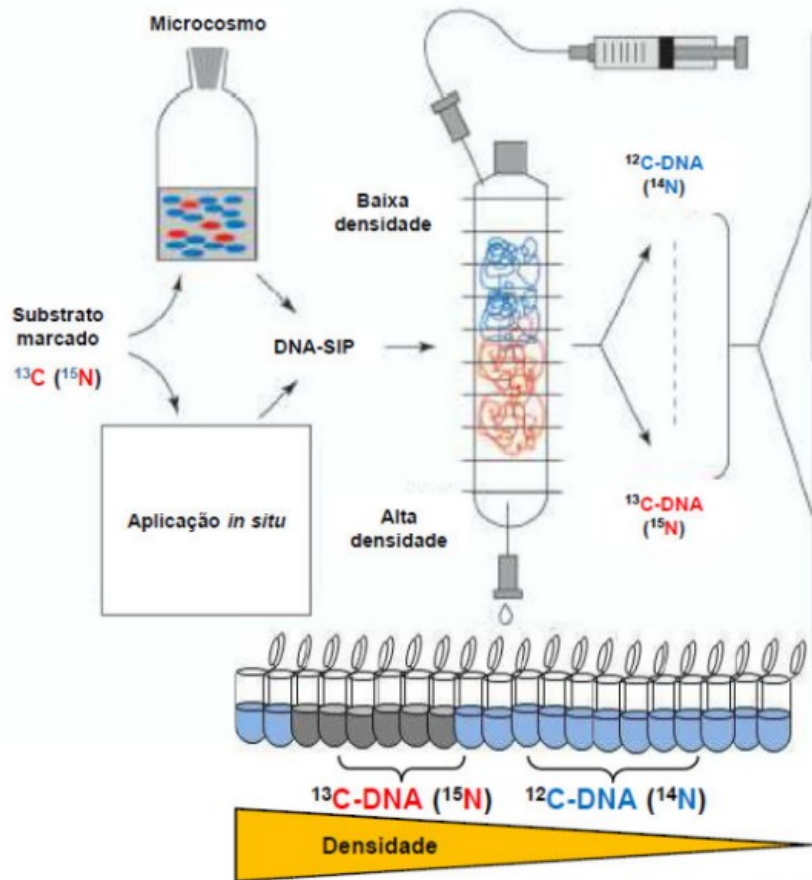




- Solution of CsCl + buffer (1,725 g/mL)
- 1 $\mu$ g of DNA



**Ultracentrífuga**  
**Beckman Optima TL**  
Velocidade: 64.000rpm  
Temperatura: 20°C  
Tempo: 36-48horas

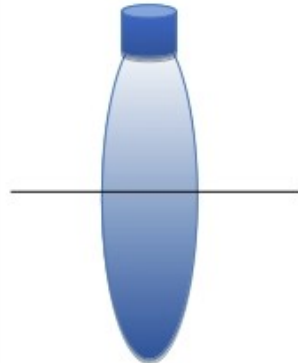
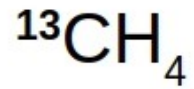
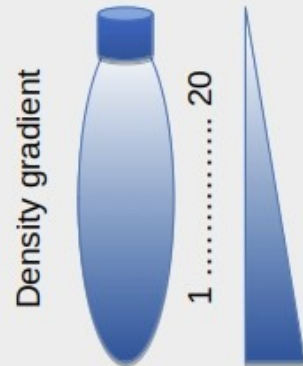
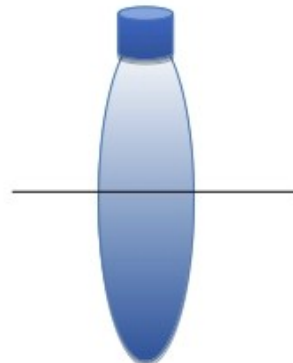
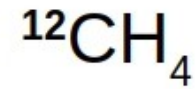


## Fractionation

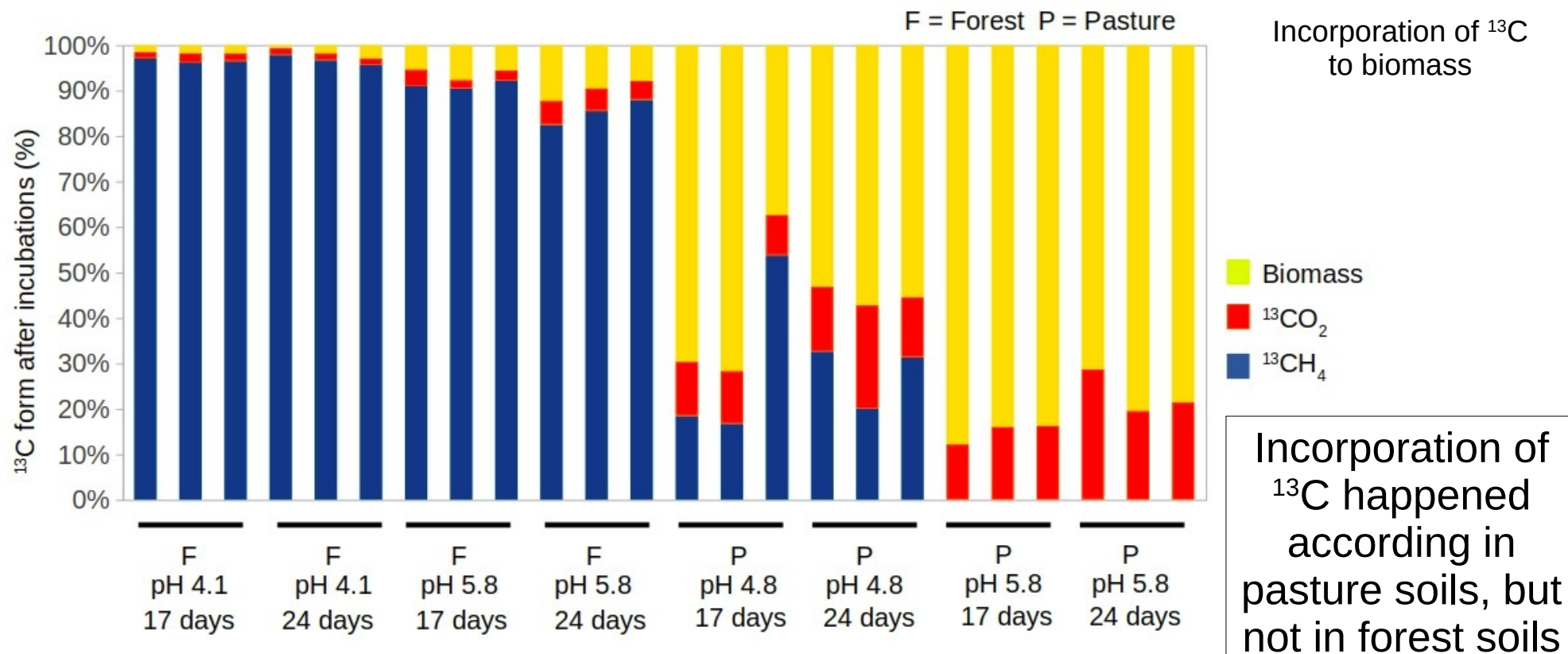
- Pump
- Blue solution (resazurine 0.1 %)
- Drops sampled at the bases, 100  $\mu\text{L}$  fractions
- Density measurement
- DNA quantification



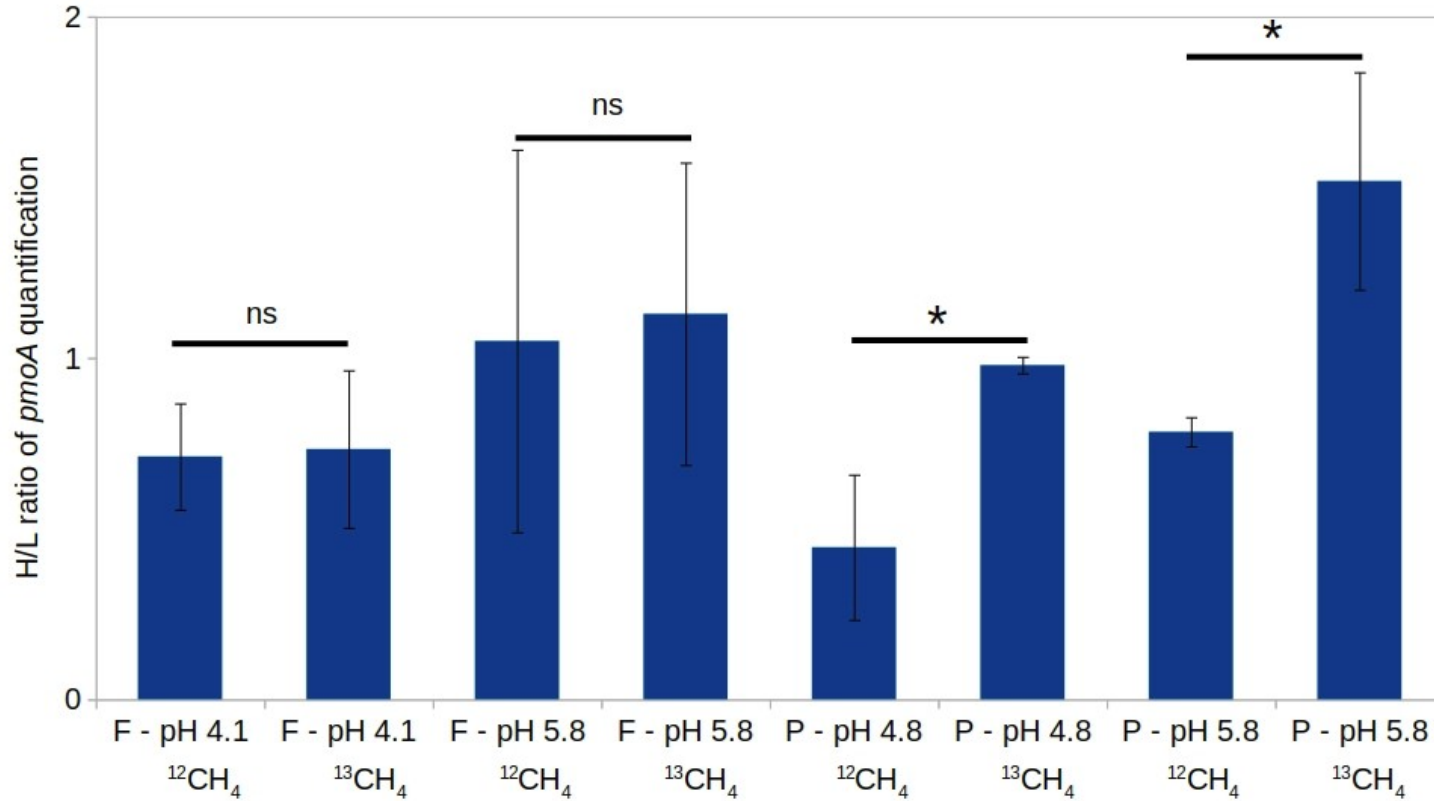
## Gradient

Incubation  
conditionsLow Density  
(L)High Density  
(H)Enrichment  
conditions

- I)  $\text{H}^{13} > \text{H}^{12}$
- e
- II)  $\text{H}^{12} \leq \text{L}^{12}$

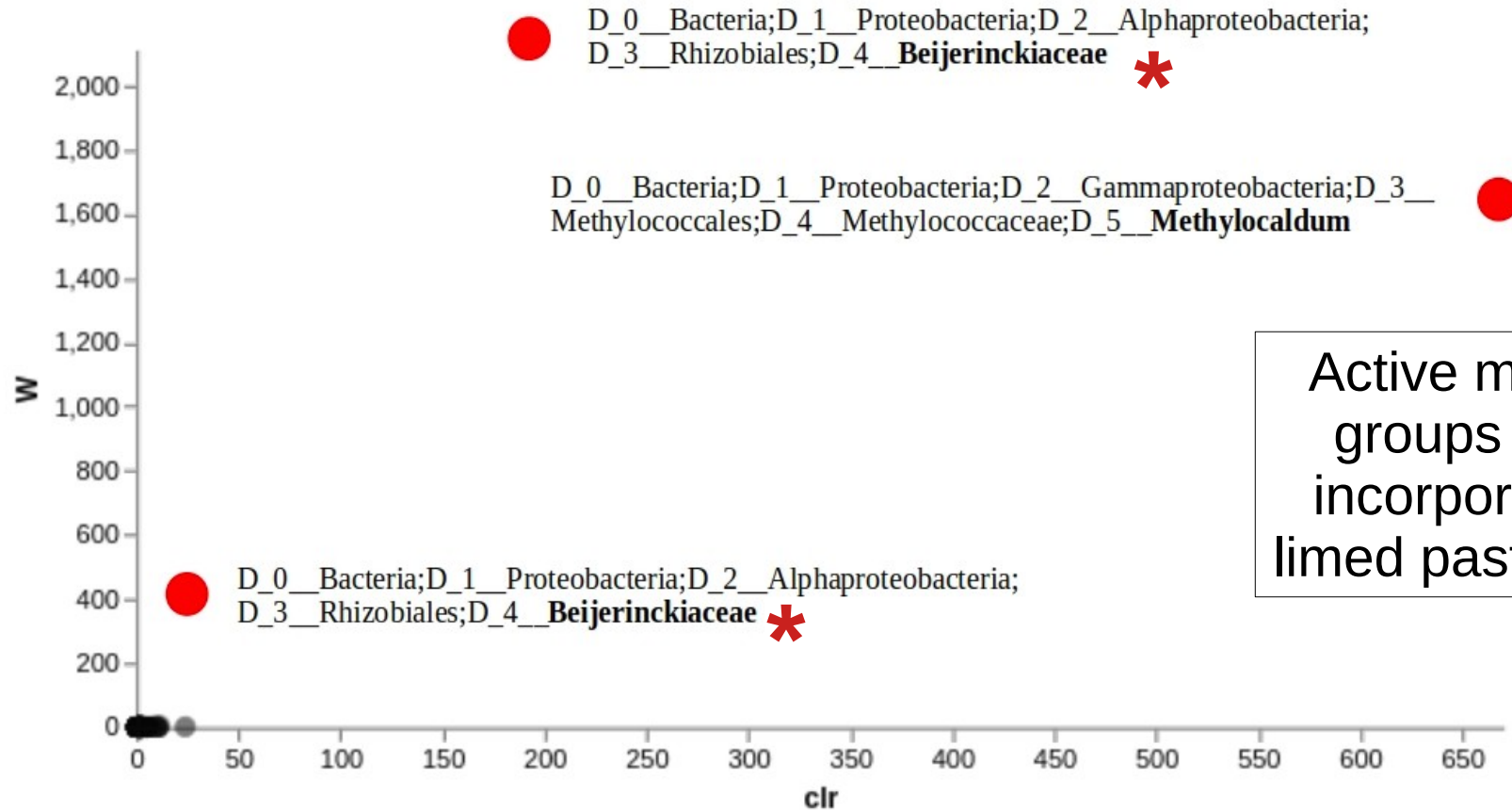




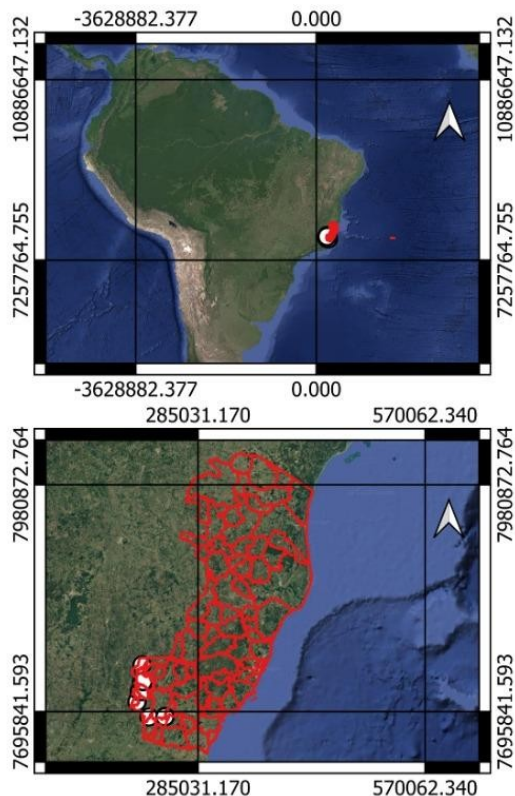


qPCR of *pmoA*  
between H/L

The abundance  
of ACTIVE  
methanotrophs  
(pMMO)  
increased only in  
pasture soils.



- ✓ Under High  $\text{CH}_4$ , *Methylocaldum* sp. (type I) e Beijerinckiaceae (type II) are active. Under atmospheric concentrations, soil sinks less methane.
- ✓ Methane sinking in forest soils depend on its acidic pH.
- ✓ Liming pastures and conserve grass coverage can mitigate soil methane emissions, depending of methane concentrations.



## Soil quality and biodiversity in Coffee cultivation comparing conventional and agroforestry systems to forest soil

- Biodiversity
- Soil fertility
- Soil biological quality





- **Target**

- Couple long-reads and short-reads to biodiversity evaluation of multiple taxonomic groups.

- **Challenges**

- Optimize methodology to use nanopore long-reads to target soil meso and macroorganisms (cluster 18S-ITS-28S)
- Create database and adjust to use with current bioinformatics amplicon tools



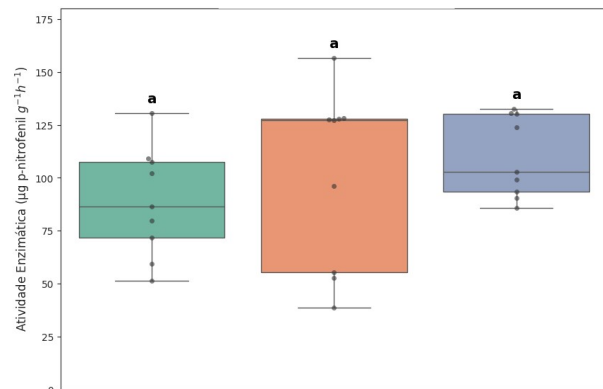
Conventional  
*Coffea arabica*



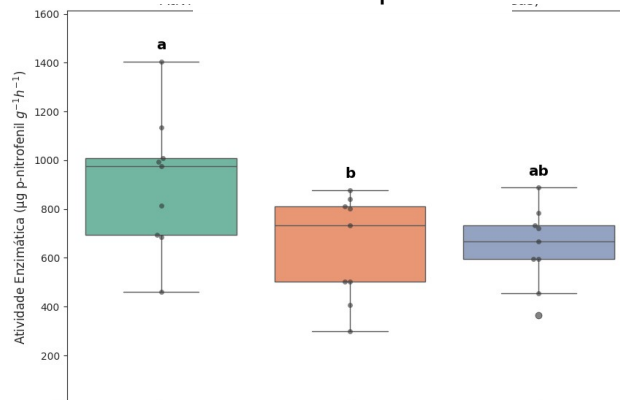
Agroforestry  
*Coffea arabica*



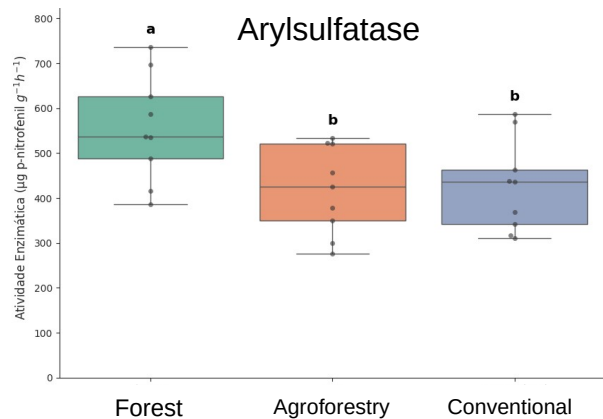
Atlantic Forest fragment

$\beta$ -glucosidase

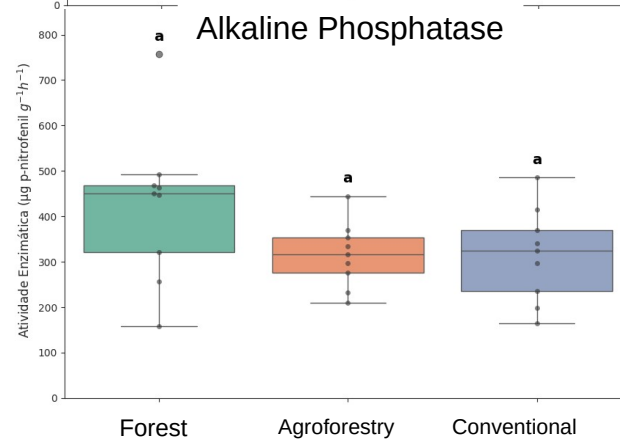
Acidic Phosphatase



Arylsulfatase



Alkaline Phosphatase





# Acknowledgments

