

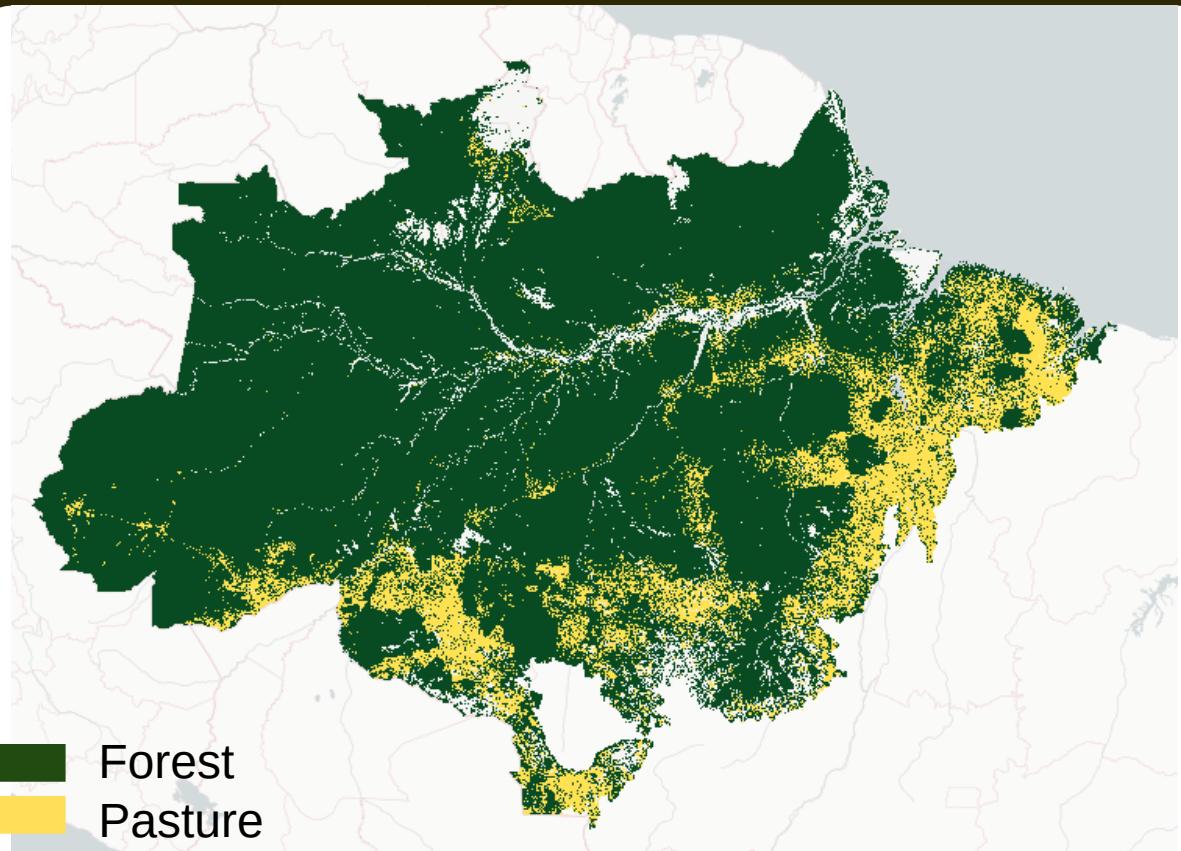
Uncovering a Hidden Methane Source: Grass Management and Soil Liming as Mitigation Strategies in Amazon Pasture Soils

Leandro Fonseca de Souza, Sc.D.

Assistant Professor

Federal University of Espírito Santo | UFES

desouza.lf@gmail.com

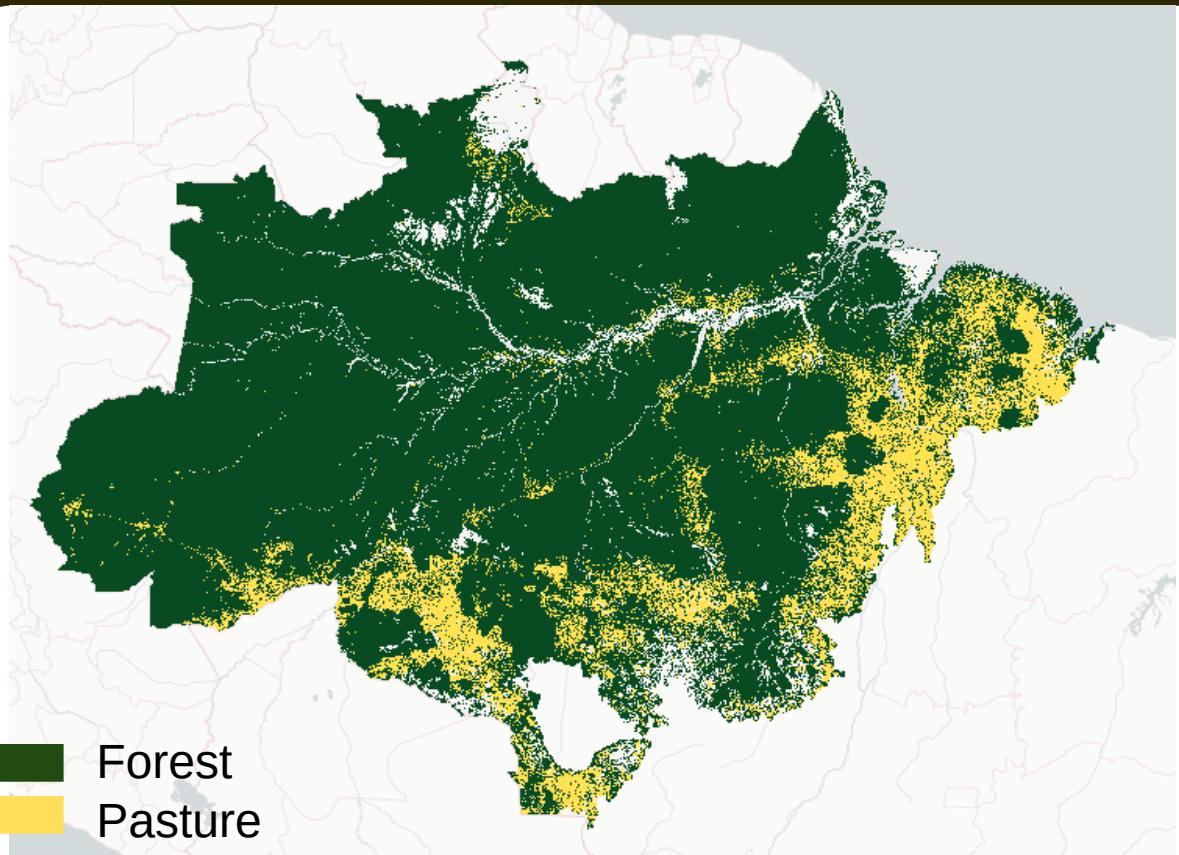


Area:
421 mi ha^a

Pastures:
56,1 mi ha^a

Degraded or under
degradation pasture:
> 30 mi ha^a

^a MapBiomas – 2024
<https://brasil.mapbiomas.org/>



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421 mi ha^a

Pastures:
56,1 mi ha^a

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> 30 mi ha^a



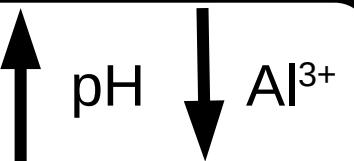
~24 mi ha

^a MapBiomas – 2024
<https://brasil.mapbiomas.org/>

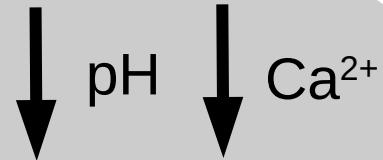
Slash and burn and soil pH



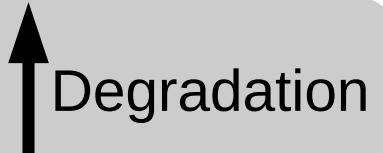
Ashes incorporation to
the soil



With time...



Without management...



Need of liming to:

- Calcium and Magnesium supply
- Restoration of degraded areas

1976 – CH₄ is a greenhouse gas

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

Emissions Of N₂O, CH₄ and CO₂
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

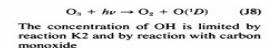
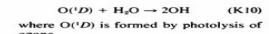
SCIENCE

**Greenhouse Effects due to Man-Made
Perturbations of Trace Gases**

Anthropogenic 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^{1(D}) (K3). The all-important hydroxyl radical involved in reaction K2 is mainly derived from



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 CH₄ fluxes in
the Brazilian Amazon Basin

Paul A. Steudler,¹ Jerry M. Melillo,¹ Brigitte J. Feigl,² Christopher Neill,¹
Marisa C. Piccolo,² and Carlos C. Cerri²



**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_4 fluxes (east)



Geoderma 107 (2002) 227–241

GEODERMA
www.elsevier.com/locate/geoderma

Seasonal variation of soil chemical properties and CO_2 and CH_4 fluxes in unfertilized and P-fertilized pastures in an Ultisol of the Brazilian Amazon

Silvana Aparecida Pavan Fernandes ^{a,*}, Martial Bernoux ^b,
Carlos C. Cerri ^a, Brigitte J. Feigl ^a, Marisa C. Piccolo ^a

Ecosystems (2000) 3: 41–56
DOI: 10.1007/s100210000009

ECOSYSTEMS
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ORIGINAL ARTICLES

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

Louis V. Verchot,^{1,2*} Eric A. Davidson,^{1,2} J. Henrique Cattânia,²
and Ilse L. Ackerman¹

2002 – Importance of seasonality in soil CH_4 fluxes (west)

frontiers
in Microbiology

ORIGINAL RESEARCH
published: 06 October 2015
doi: 10.3389/fmicb.2015.01057

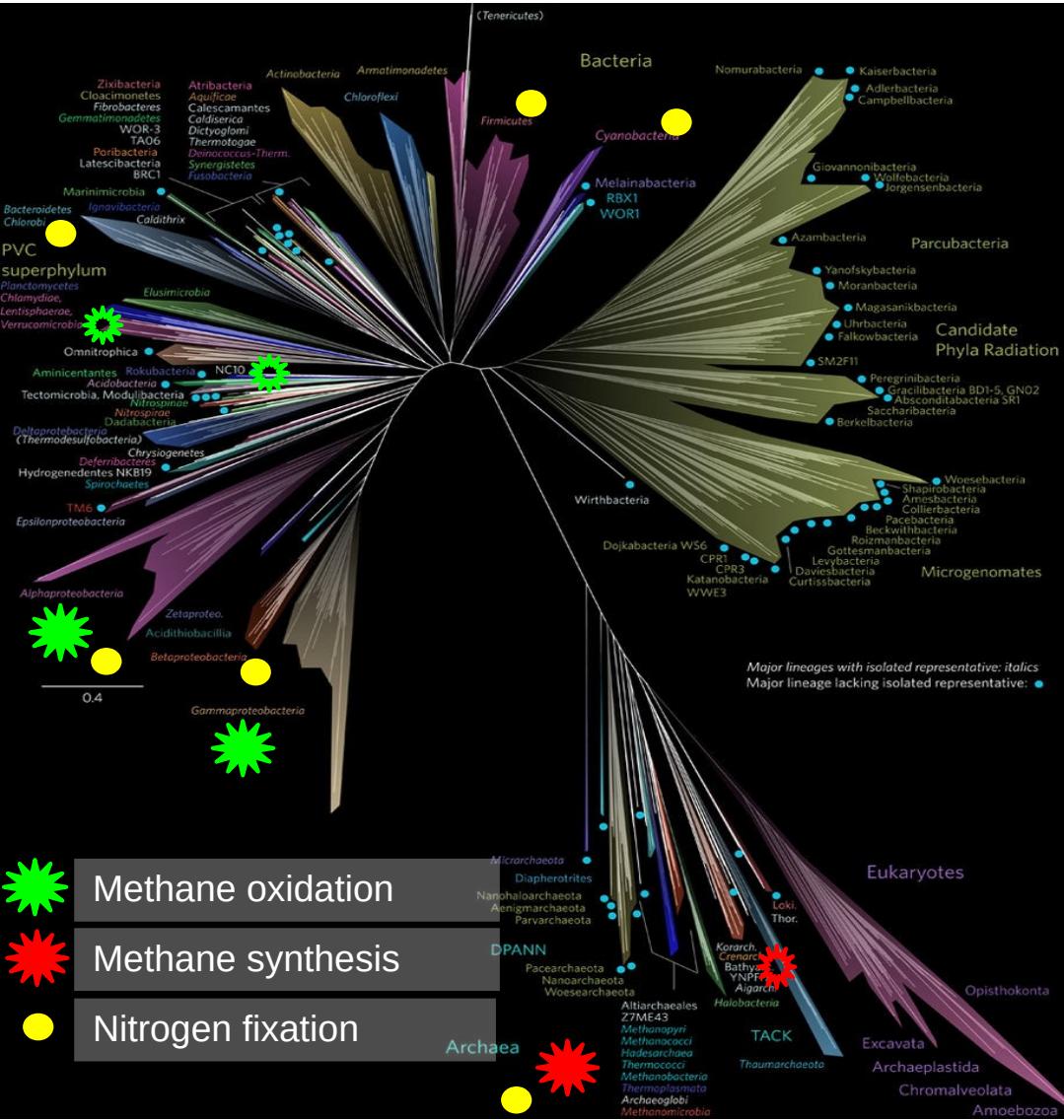


Specific microbial gene abundances and soil parameters contribute to C, N, and greenhouse gas process rates after land use change in Southern Amazonian Soils

OPEN ACCESS
Edited by:
Silvia Pajares Moreno,

Daniel R. Lammel^{1,2*}, Brigitte J. Feigl¹, Carlos C. Cerri¹ and Klaus Nüsslein^{2*}

2015 – First insights on Amazon soil methane microbiota



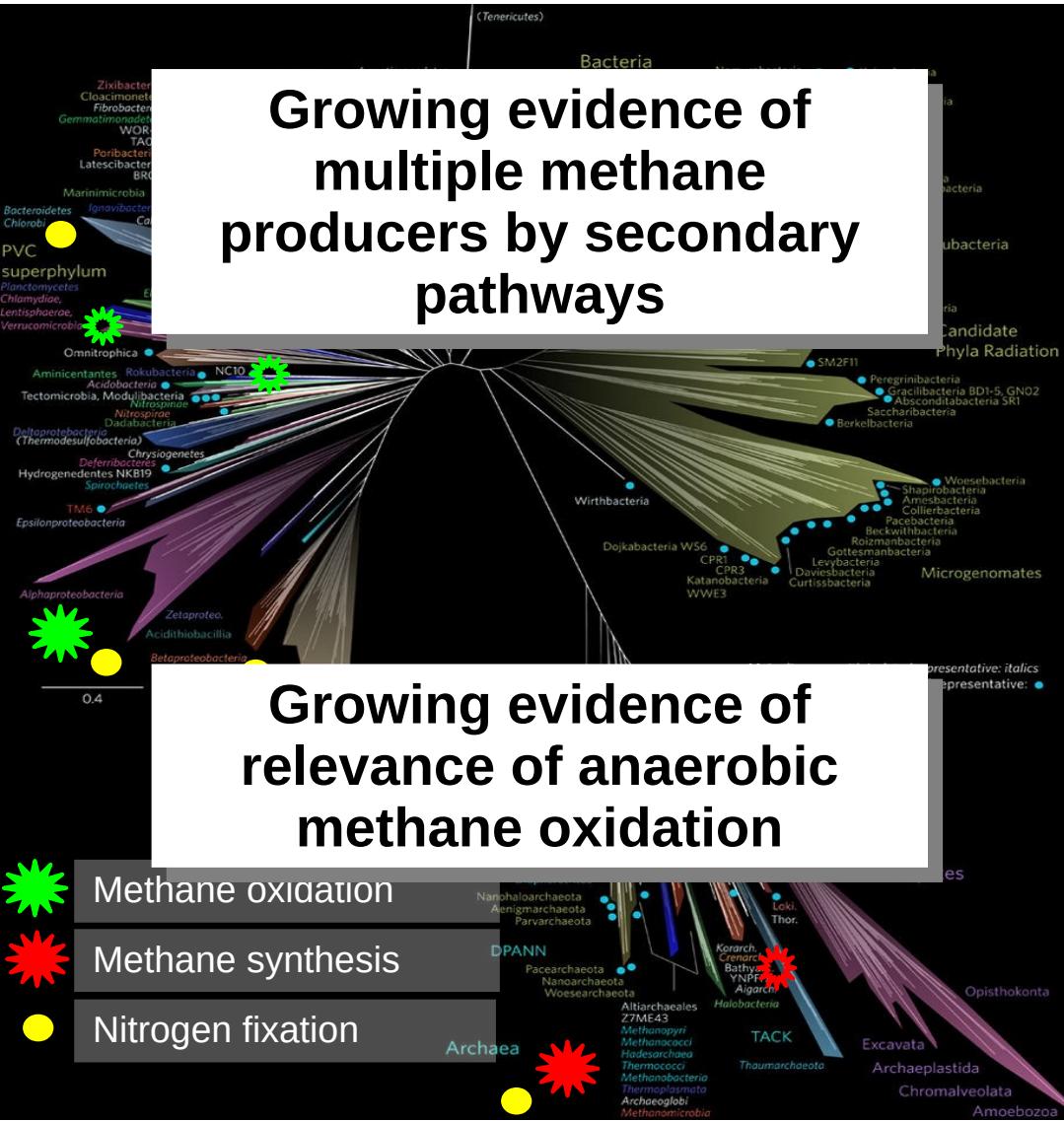
Methane producers and consumers (best characterized)

Methanogens

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

Methanotrophs

- ✓ **Alfaproteobactérias**
 - ✓ Methylocystaceae
 - ✓ Beijerinckiaceae
- ✓ **Gamaproteobactérias**
 - ✓ Methylococcaceae
 - ✓ Methylothermaceae
 - ✓ Crenotrichaceae
- ✓ **Verrucomicrobia**
 - ✓ Methylacidiphilaceae



Methane producers and consumers (best characterized)

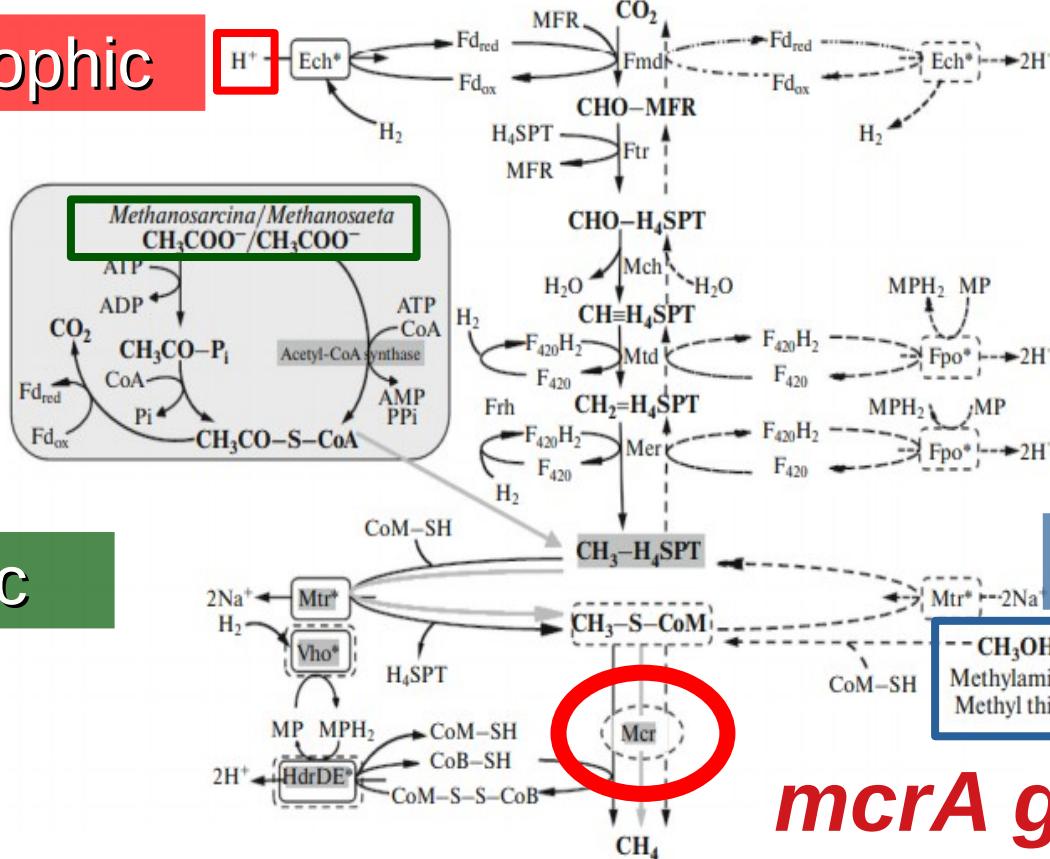
Methanogens

- ✓ Euryarchaeota
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Methanotrophs

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- ✓ Gamaproteobactérias
 - ✓ Methylococcaceae
 - ✓ Methylothermaceae
 - ✓ Crenotrichaceae
- ✓ Verrucomicrobia
 - ✓ Methylacidiphilaceae

Hidrogenotrophic



Acetoclastic

Methanotrophic

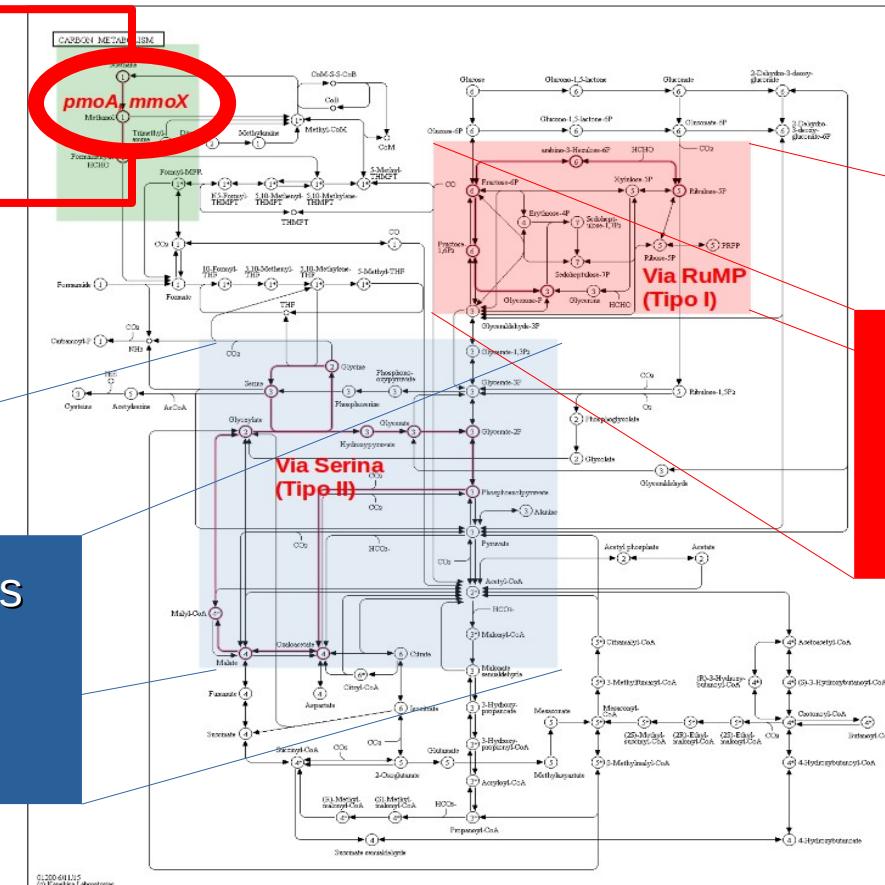
mcrA gene

Methane

Methanol

Type II Methanotrophs
Alfaproteobacteria

Serines pathway

Type I Methanotrophs
Gammaproteobacteria

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



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



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

^a Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP, Brazil

^b Department of Microbiology, University of Massachusetts, Amherst, MA, USA

^c Applied Soil Ecology Lab, School of Environmental Sciences, University of Guelph, Guelph, ON, Canada

^d Department of Evolutionary Biology and Environmental Studies, University of Zurich, Zurich, Switzerland

^e Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP, Brazil

^f Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

^g Department of Land, Air and Water Resources, University of California Davis, CA, USA

Experiment 1

Amazon West
~250 dias



Experiment 2

Amazon East
~80 dias



Methodology - Study 1

Sequencing 16S rRNA

qPCR – *pmoA* and *mcrA*

CH_4 fluxes

Field Study

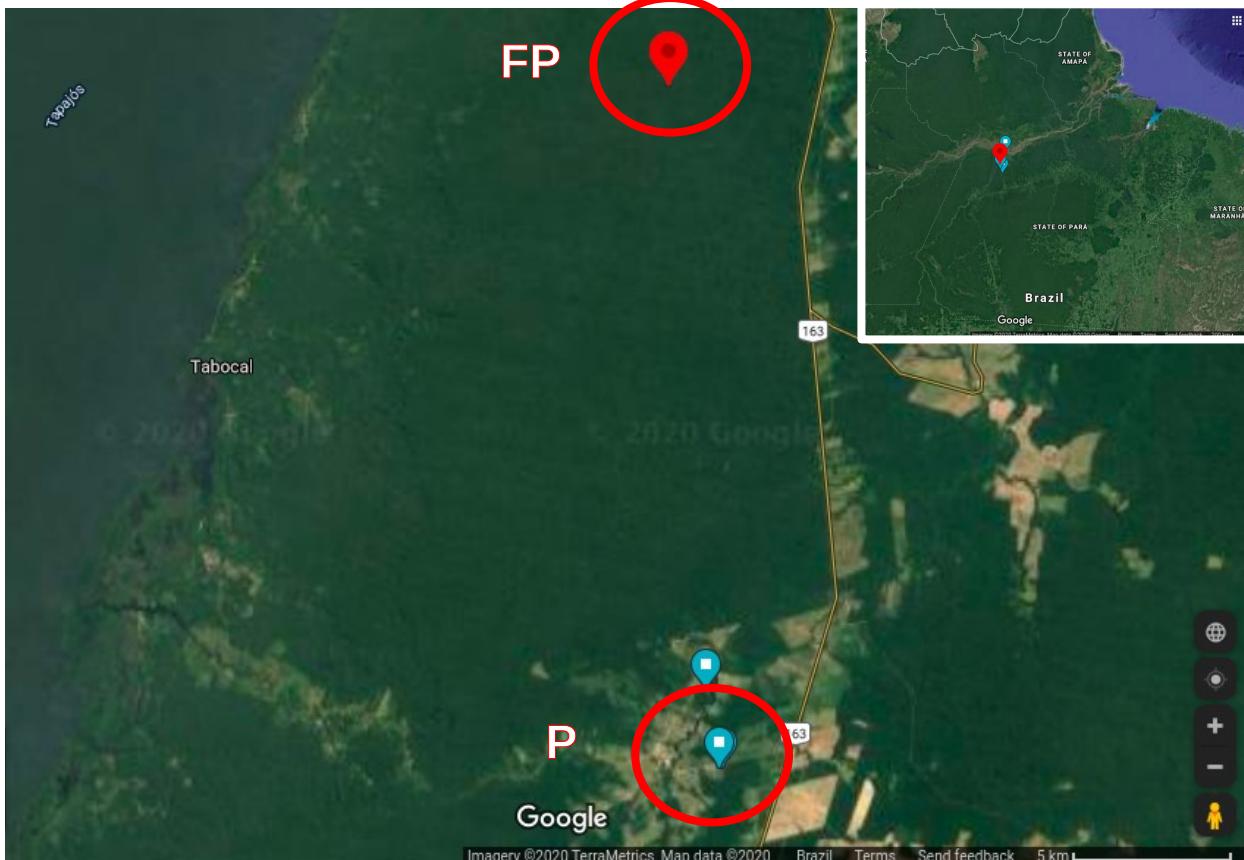
Belterra/PA





Ariquemes/RO Amazon West

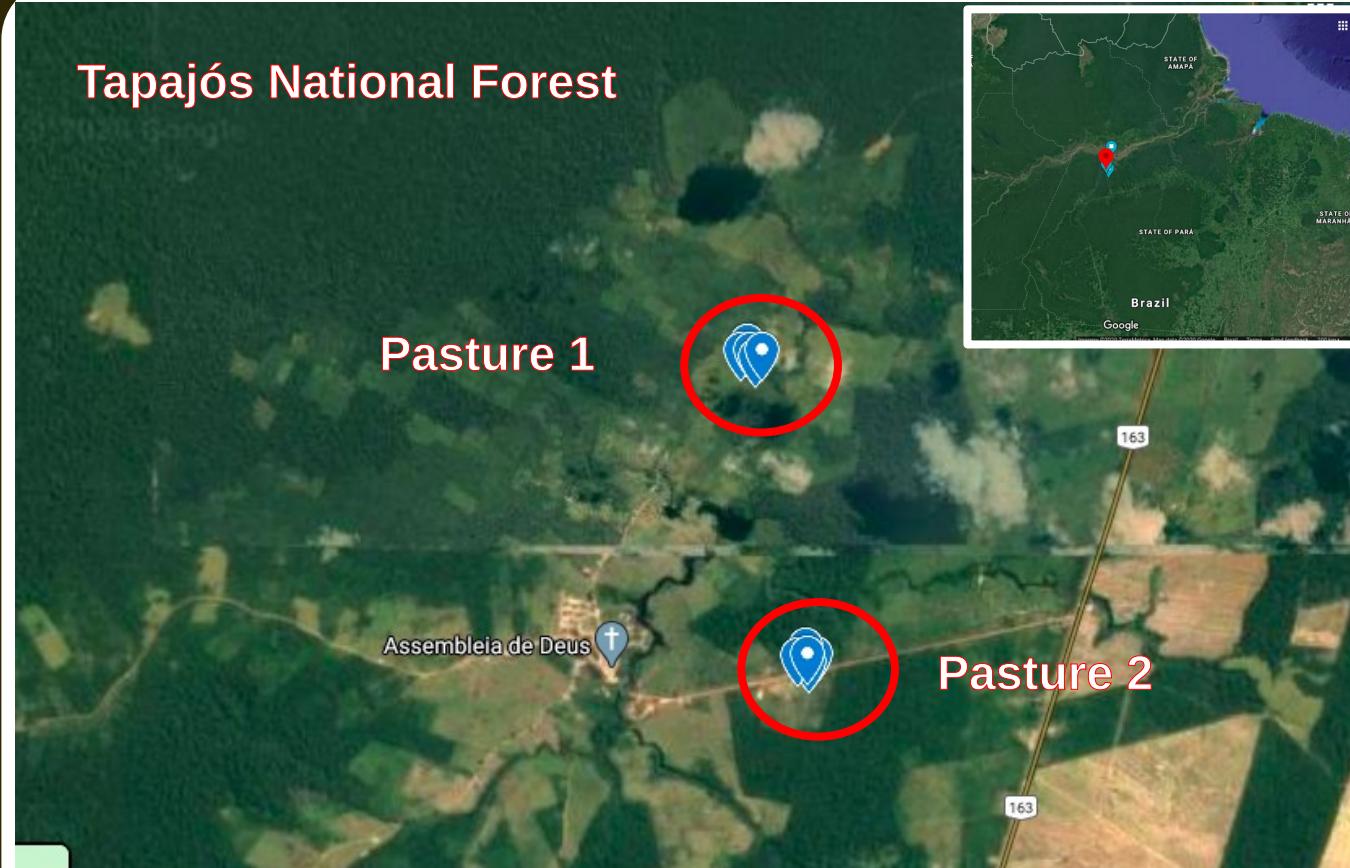
- Pastureland – 1972
- Fazenda Nova Vida
- Forest fragment
- Smallholding
- Soil type: Yellow–red Oxisols
- Texture: medium clay to sandy
- Sampling (0–10 cm): Apr/2017 (rainy season)
- Transect sampling: 5 points, 50 m apart



Belterra-Santarém/PA Amazon East

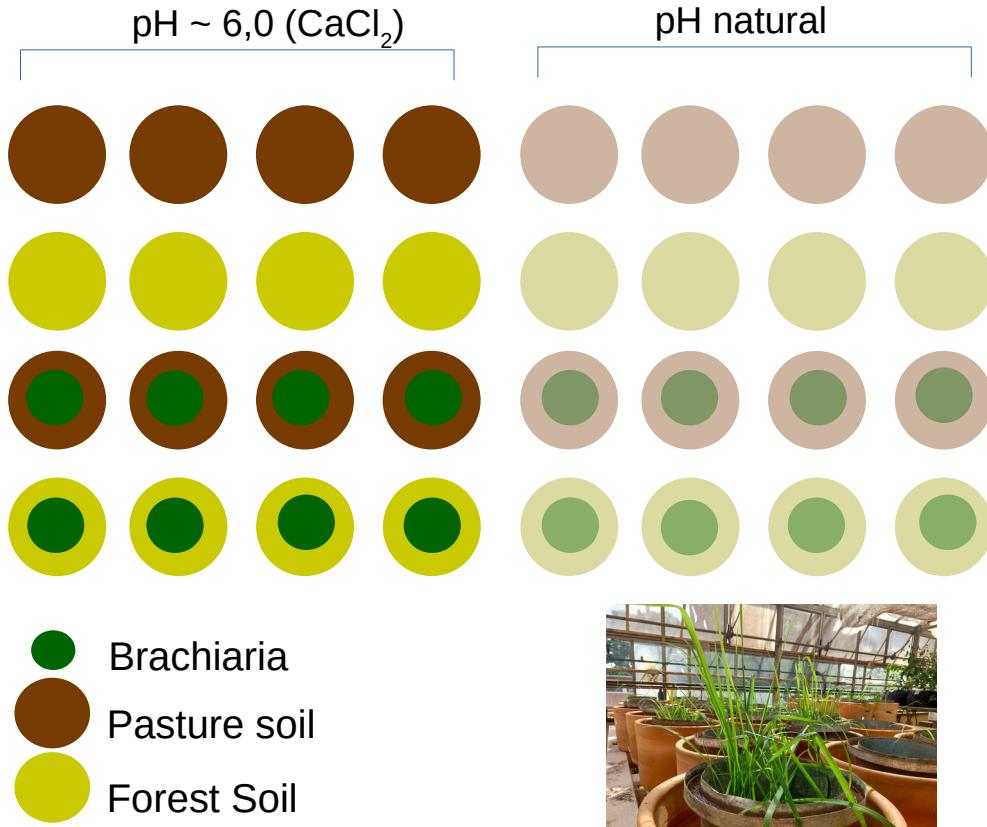
- Pastureland – 1989–1994
- Smallholding
- National forest
- Conservation area
- Texture: medium clay to sandy
- Sampling (0–10 cm): Aug/2019 (end of the rainy season)
- Transect sampling: 5 points, 50 m apart

Tapajós National Forest



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

- ✓ CH₄, CO₂, 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

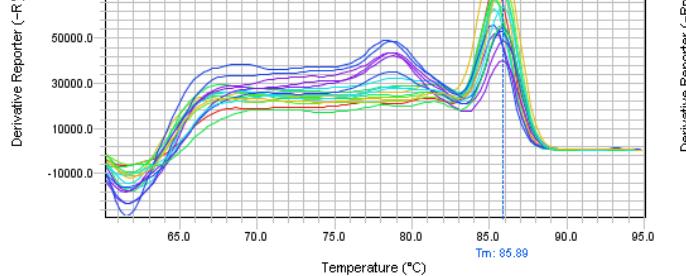


Laser spectroscopic analyser (ABB, Switzerland)

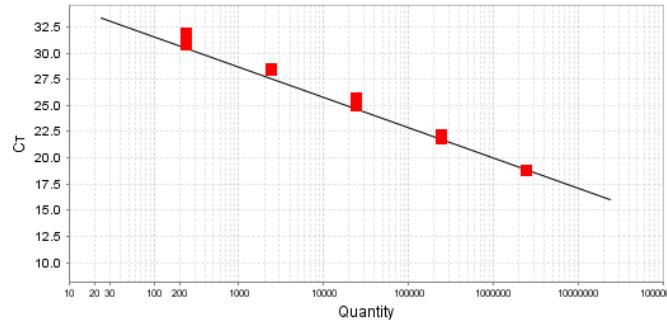
Methodology - qPCR

*mcrA*

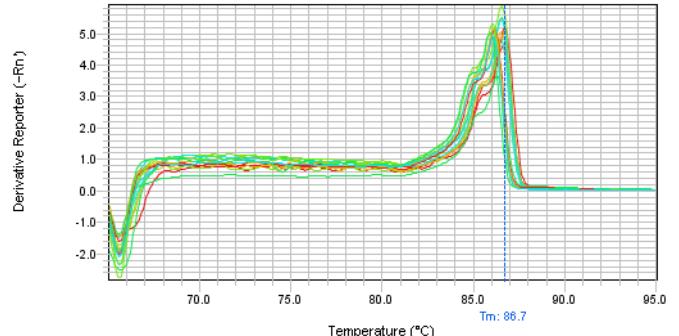
Melt Curve



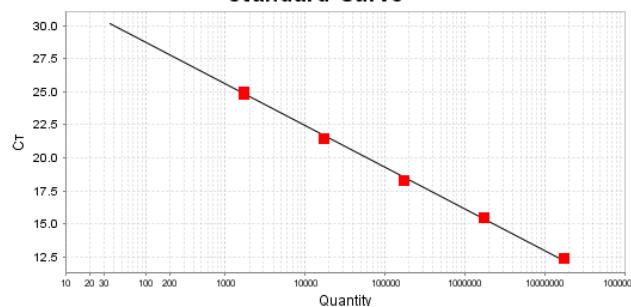
Standard Curve

*mmoX*

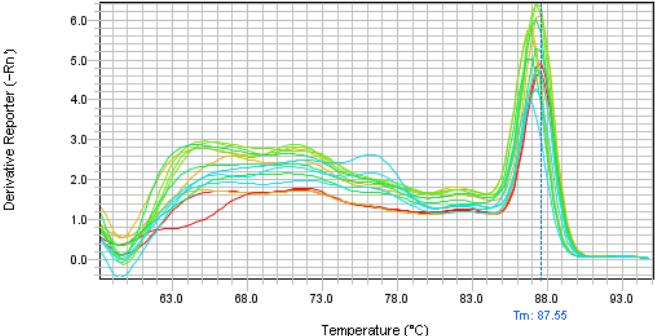
Melt Curve



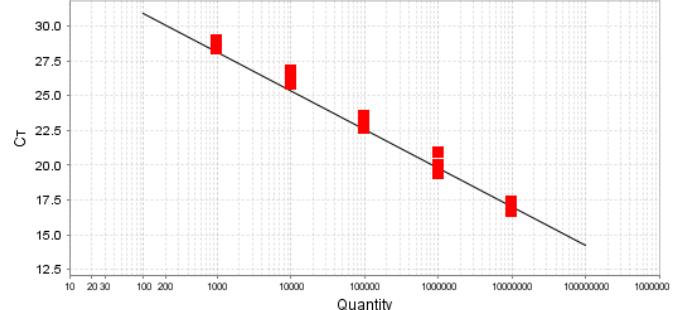
Standard Curve

*pmoA*

Melt Curve



Standard Curve



* linreg

Sequencing

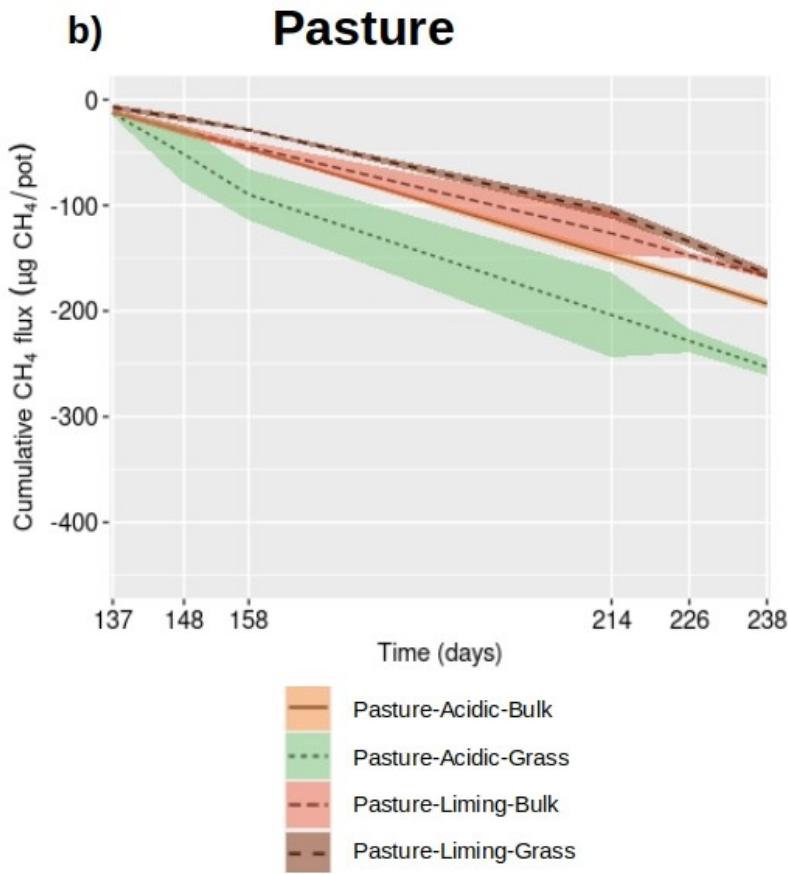
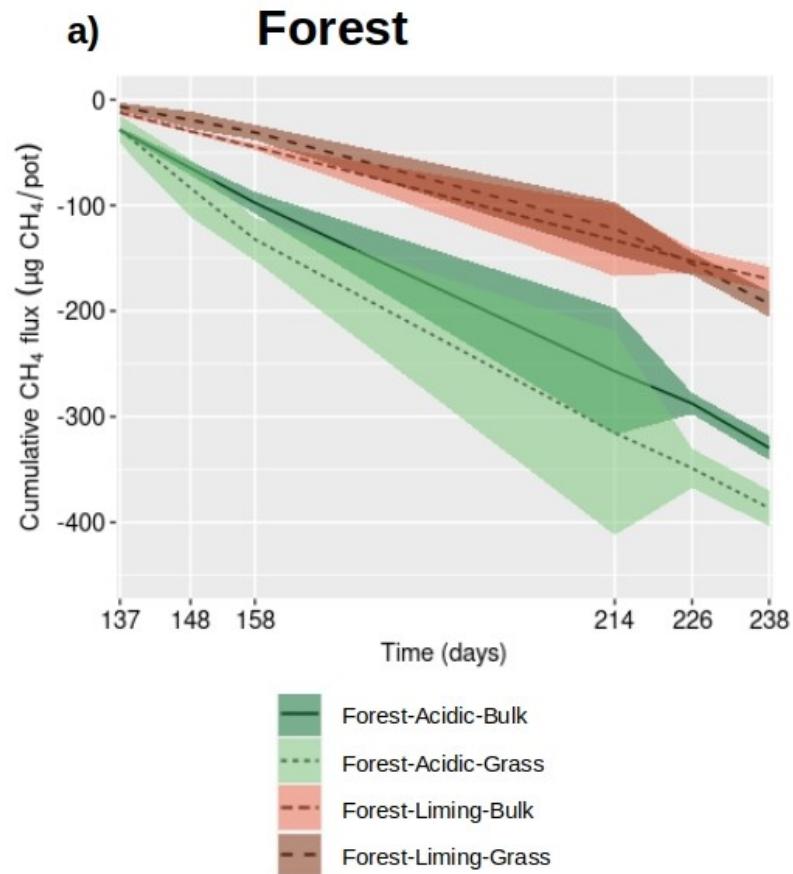
- University of Sao Paulo
- Illumina MiSeq v3 (2x250pb)
- *Paired-end*
- 16S rRNA (v4)
- 515F (Parada)/ 806R (Apprill)

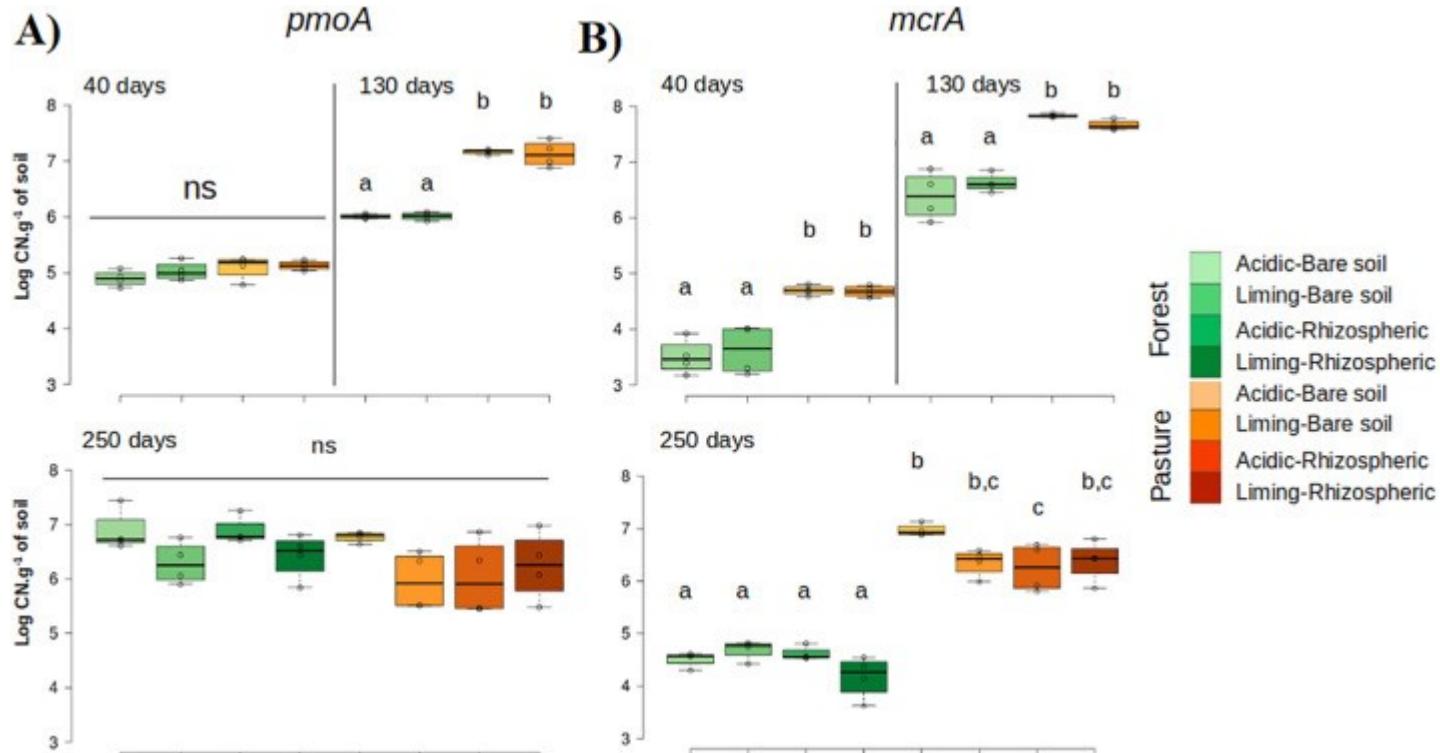


Data processing

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

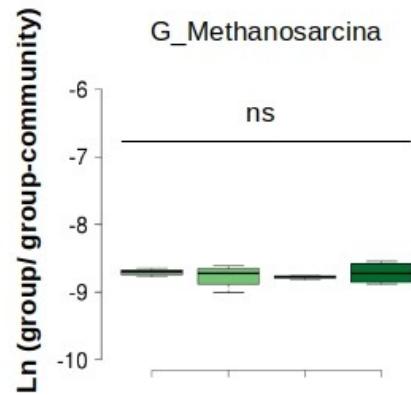






- *mcrA* is more abundant in pasture soils
- *pmoA* tends to decrease in forest soils under liming (ns)
- *mcrA* is significantly reduced in the rhizosphere of pasture soils
- *mmoX* shows no significant changes

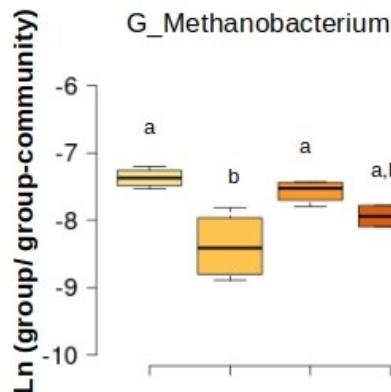
Forest



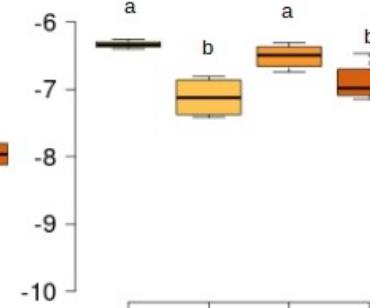
Forest Pasture

Acidic-Bare soil
Acidic-Rhizospheric
Liming-Bare soil
Liming-Rhizospheric
Acidic-Bare soil
Acidic-Rhizospheric
Liming-Bare soil
Liming-Rhizospheric

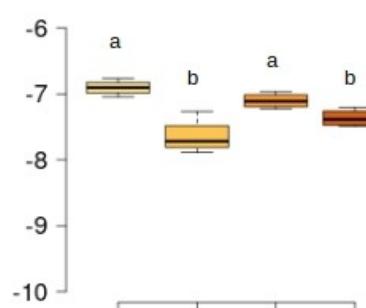
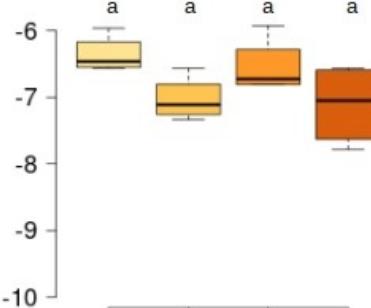
Pasture

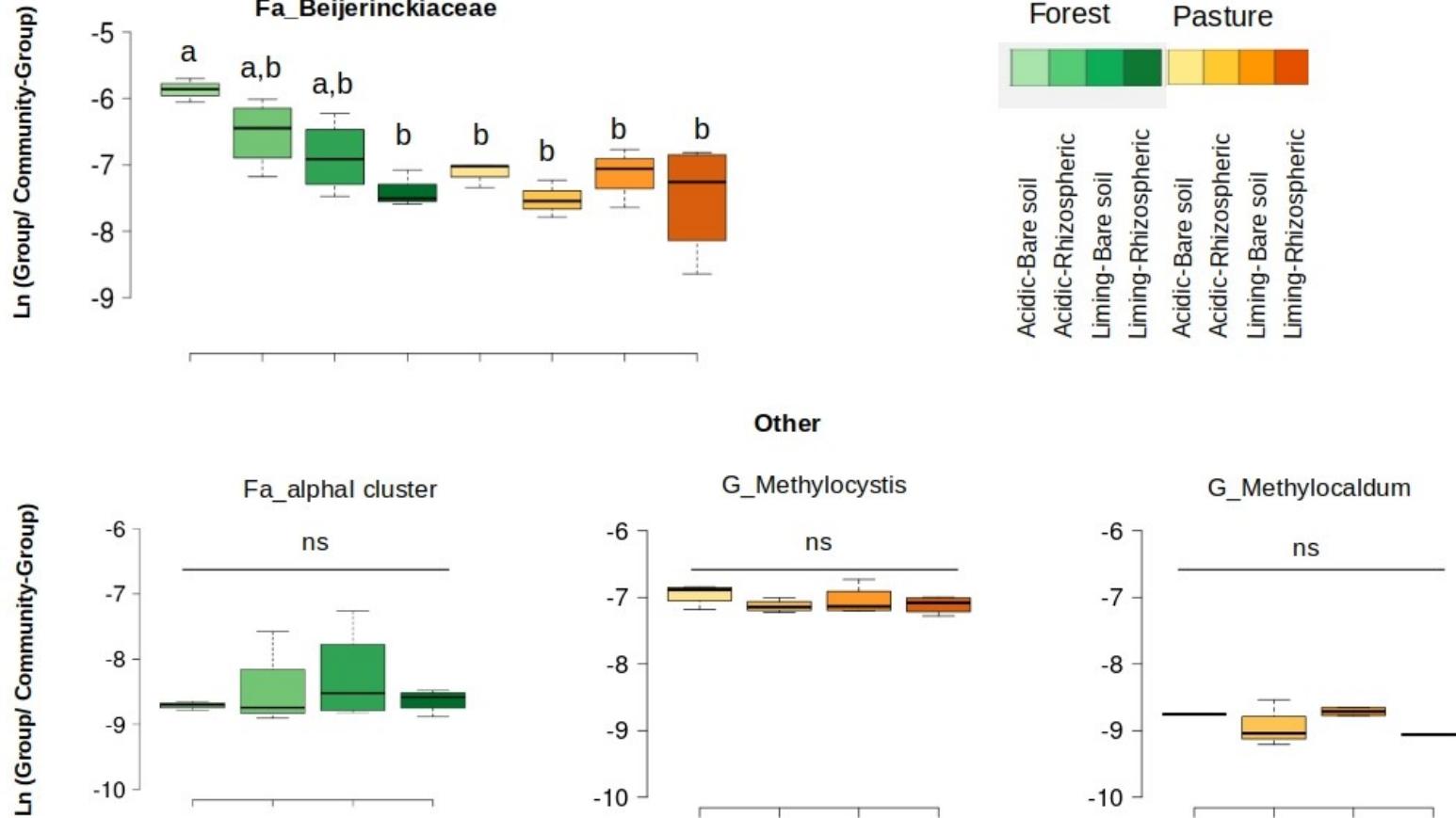


G_Methanocella



G_Rice Cluster I

G_Methanosa*cina*



- 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 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), Ixae303

<https://doi.org/10.1093/jambo/ixaе303>

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 ^{1,2,3,*}, Fernanda Mancini Nakamura ^{1,4}, Marie Kroeger ⁵,
Daniel Obregon ^{1,6}, Moacir Tuzzin de Moraes ^{1,7}, Mariana Gomes Vicente ¹,
Marcelo Zacharias Moreira ¹, Vivian Helena Pellizari ⁴, Siu Mui Tsai ¹, Klaus Nüsslein ³

¹Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP 13400-970, Brazil

²Center for Agricultural Sciences and Engineering, Federal University of Espírito Santo, Alegre, ES 29500-000, Brazil

³Department of Microbiology, University of Massachusetts, Amherst, MA 01003-9298, USA

⁴Oceanographic Institute, University of São Paulo, São Paulo, SP 05508-120, Brazil

⁵Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM 87545-1663, USA

⁶School of Environmental Sciences, University of Guelph, Guelph, ON N1G 2W1, Canada

⁷Department of Soil Science, Luiz de Queiroz College of Agriculture, University of São Paulo, Piracicaba, SP 13418-900, Brazil

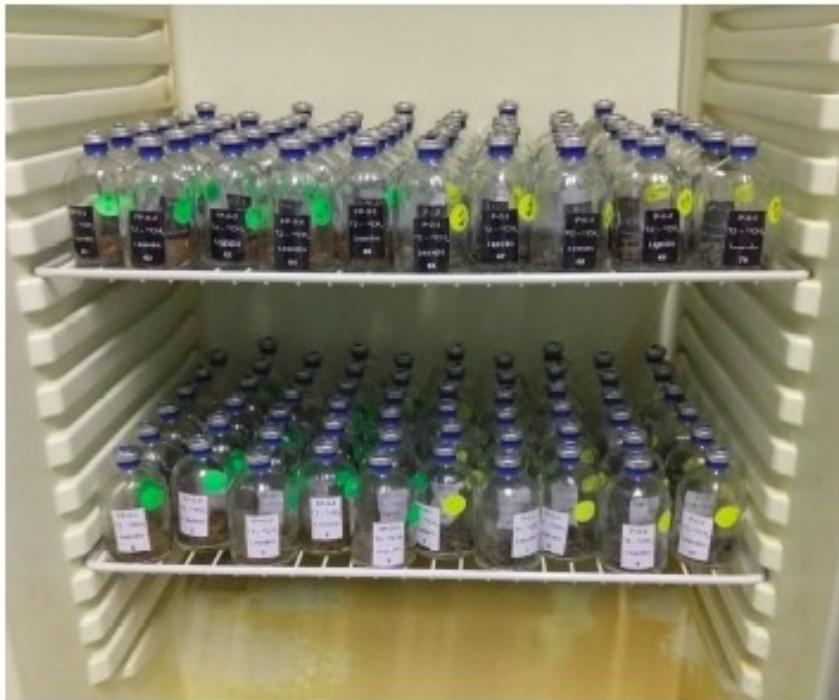
*Corresponding author. Center for Nuclear Energy in Agriculture, University of São Paulo, Piracicaba, SP 13400-970, Brazil.

E-mail: leandro_fonseca@alumni.usp.br



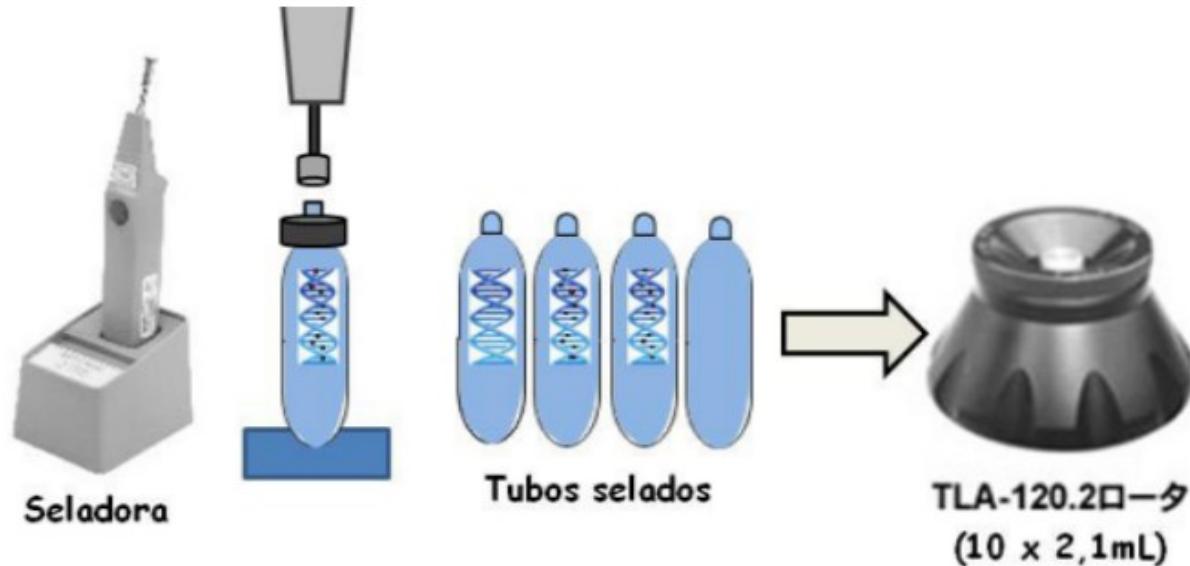
Experiment design

- 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
- CH_4 flux measurements: weekly



Experiment

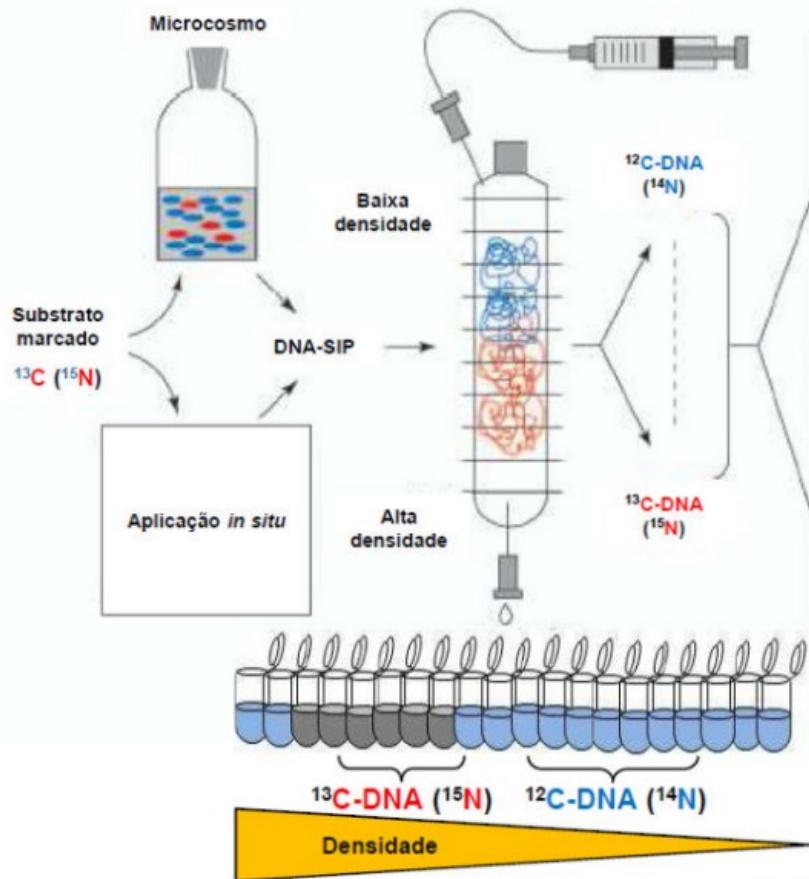
- ✓ Microcosms sealed with rubber lids
 - 120 mL
 - 10 g of soils
 - Temperature: 25 °C BOD
- ✓ Moisture: 70-80%
- ✓ Concentration of $^{13}\text{CH}_4$
 - ~10,000 ppm
- ✓ 24 days
- ✓ Readings and replacement of $^{13}\text{CH}_4$ every 2-4 days



- Solution of CsCl + buffer (1,725 g/mL)
- 1µg of DNA



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



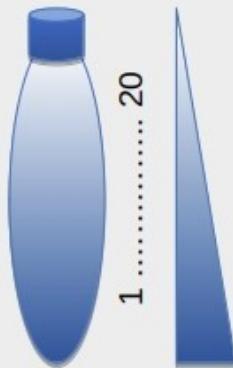
Fractionation

- Pump
- Blue solution (resazurine 0.1 %)
- Drops sampled at the bases, 100 μL fractions
- Density measurement
- DNA quantification

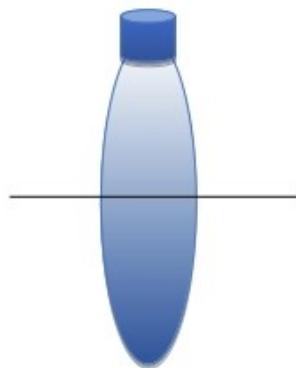


Gradient

Density gradient



$^{13}\text{CH}_4$

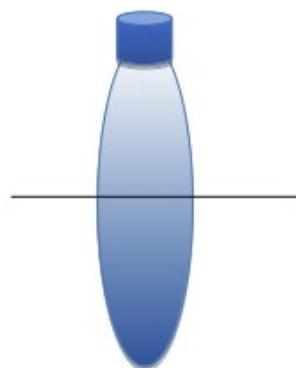


Incubation conditions

Low Density (L)

High Density (H)

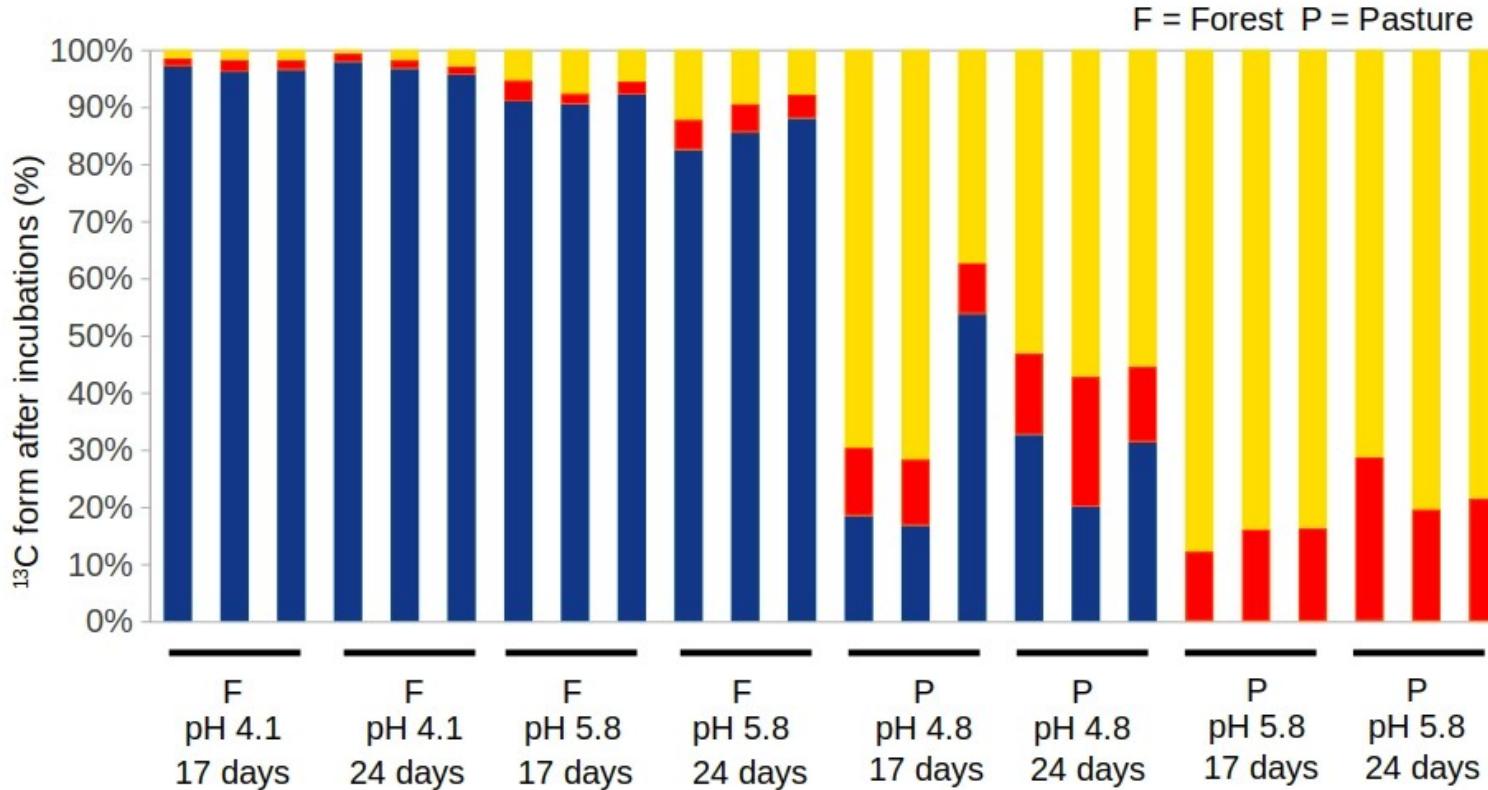
$^{12}\text{CH}_4$



Enrichment conditions

I) $\text{H13} > \text{H12}$
e

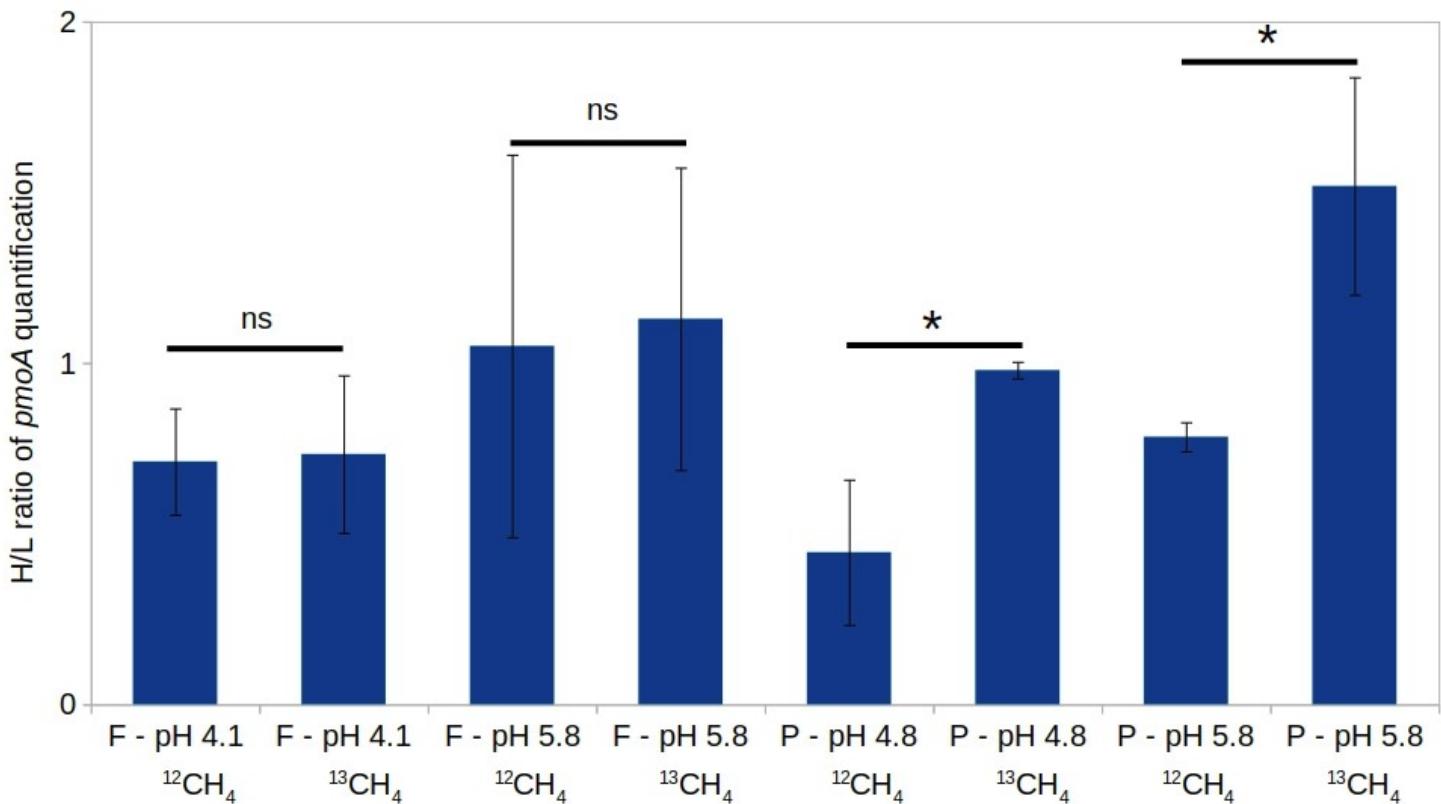
II) $\text{H12} \leq \text{L12}$



Incorporation of ^{13}C to biomass

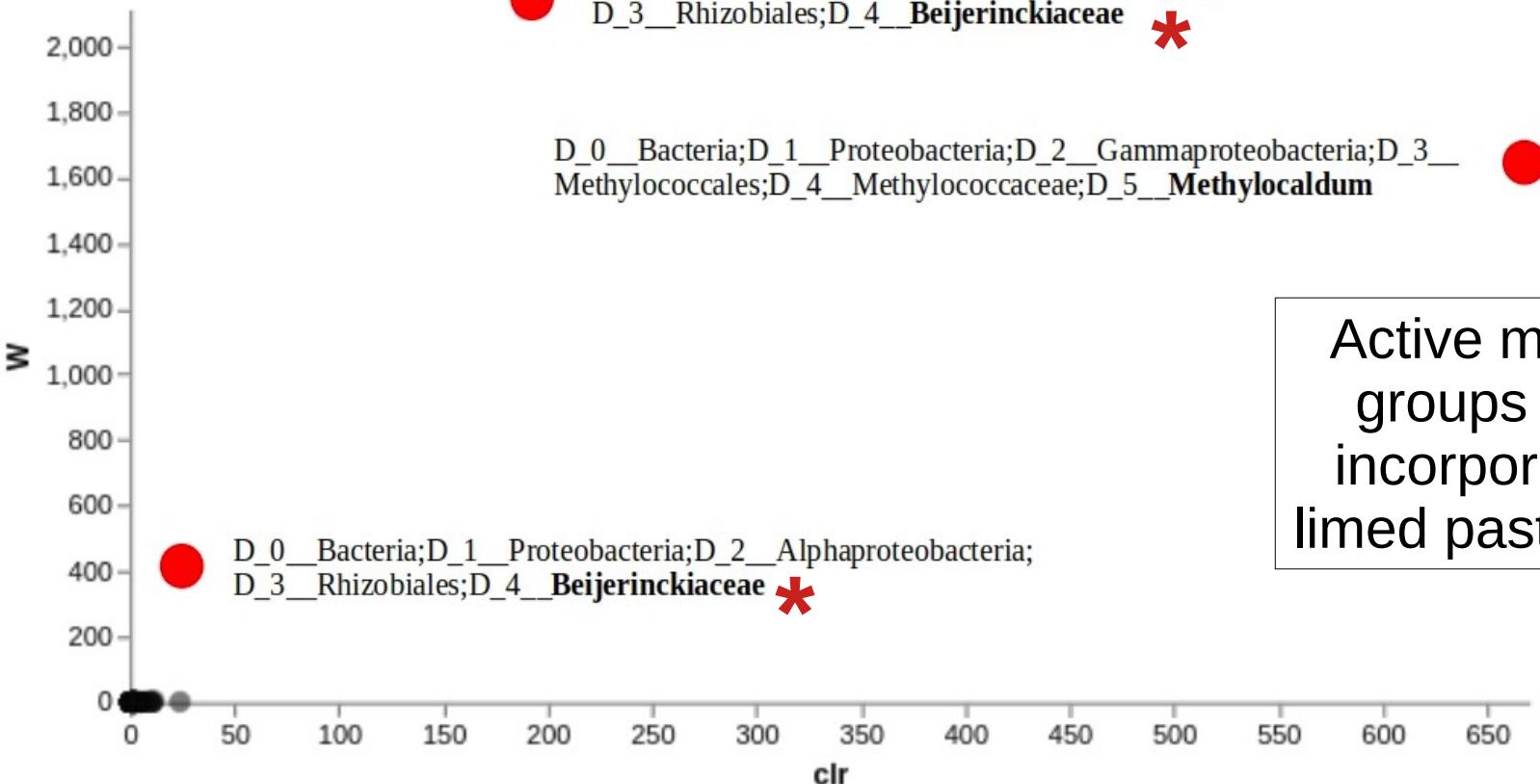
Biomass
 $^{13}\text{CO}_2$
 $^{13}\text{CH}_4$

Incorporation of ^{13}C happened according in pasture soils, but not in forest soils



qPCR of *pmoA*
between H/L

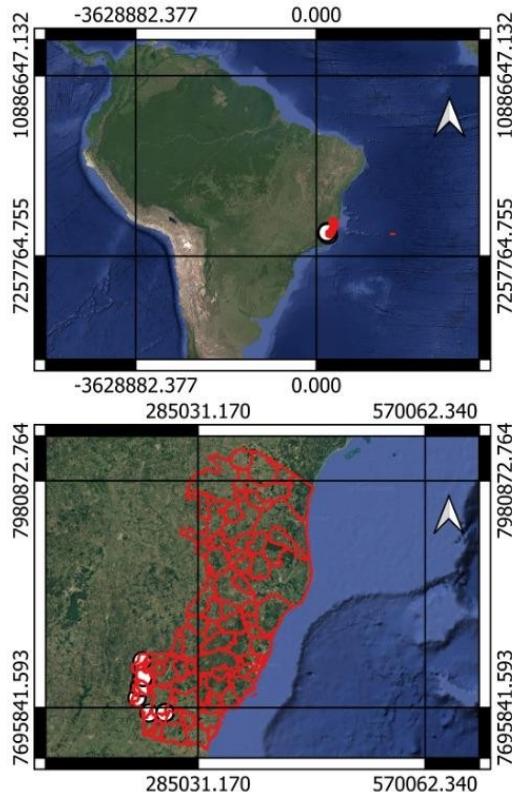
The abundance
of ACTIVE
methanotrophs
(pMMO)
increased only in
pasture soils.



Active microbial groups on ^{13}C incorporation in limed pasture soils

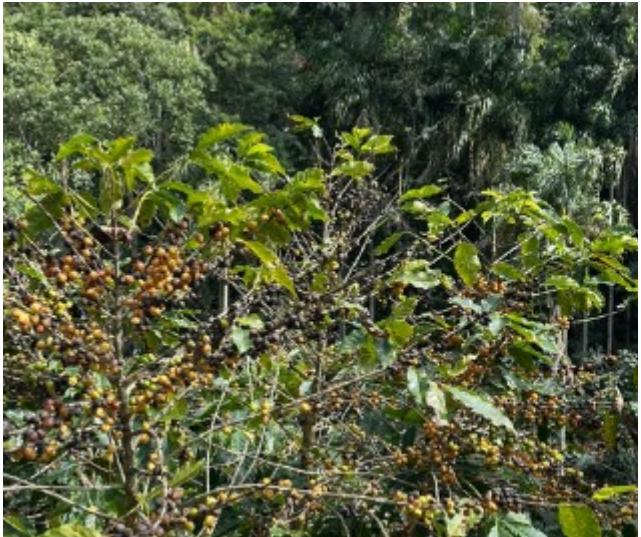
- ✓ Under High CH₄, *Methylocaldum* sp. (type I) e *Beijerinckiaceae* (type II) are active. Under atmospheric concentrations, soil sinks less methane.
- ✓ Methane sinking in forest soils depend on its acidic pH.
- ✓ Liming pastures and conserve grass coverage can mitigate soil methane emissions, depending of methane concentrations.

Last research



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

- Biodiversity
- Soil fertility
- Soil biological quality

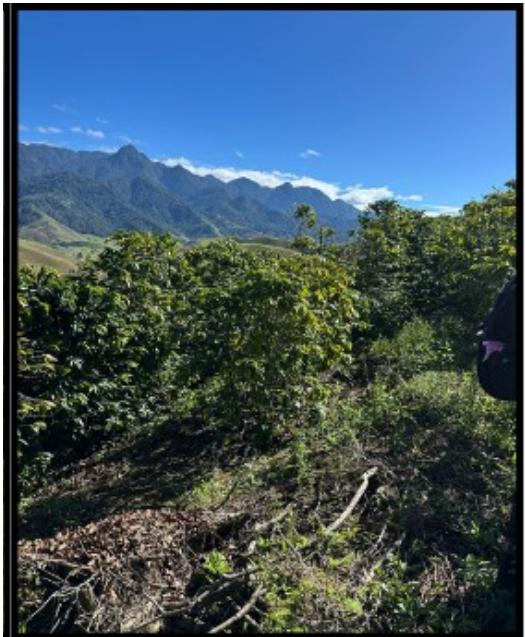


- **Target**

- 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



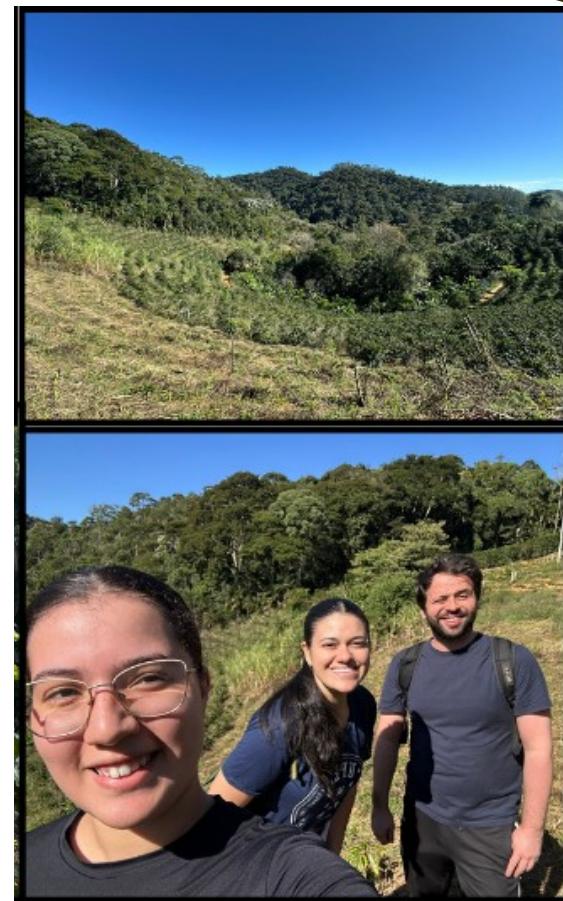
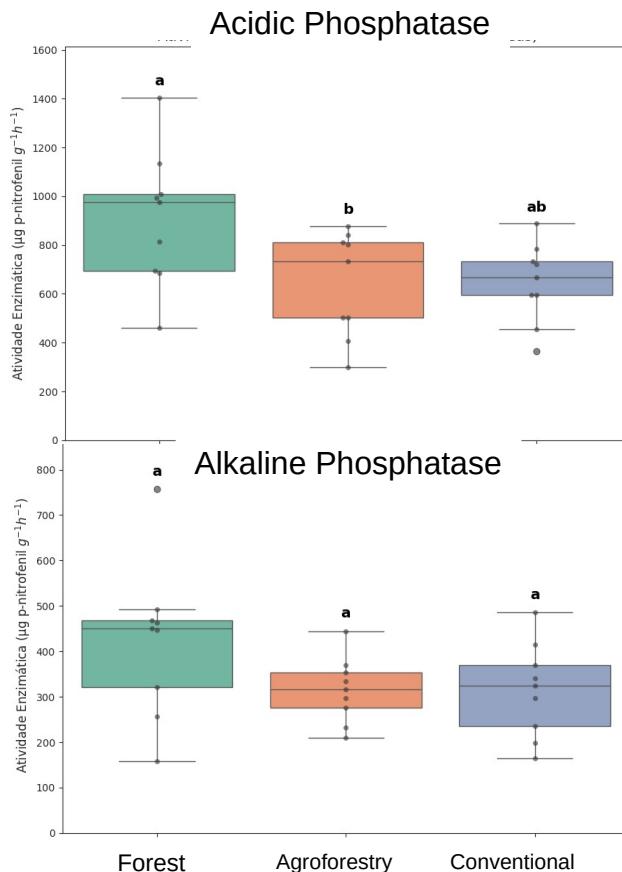
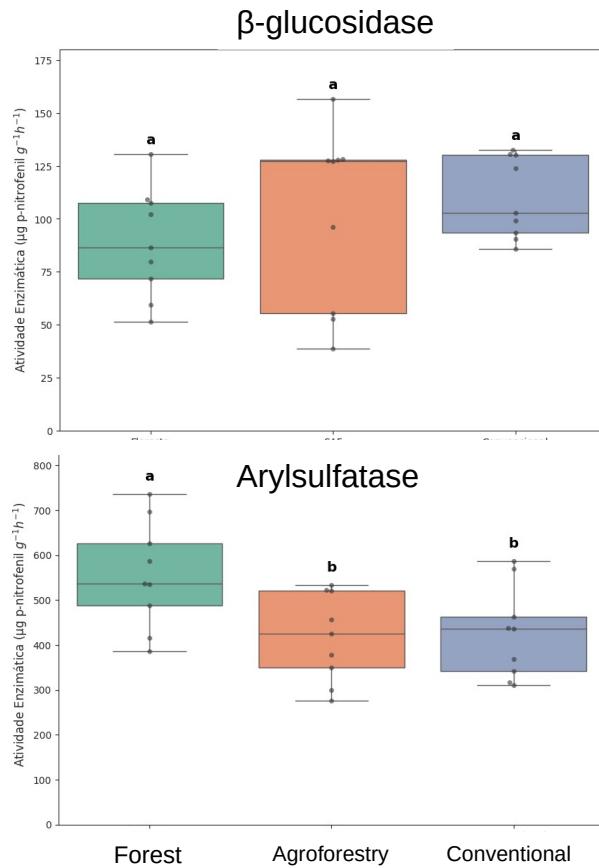
Conventional
Coffea arabica



Agroforestry
Coffea arabica



Atlantic Forest fragment



Acknowledgments

