Maintaining grass coverage increases methane uptake in Amazonian pasture soils

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Running Title: Grass rhizosphere drives soil methane cycle

Abstract

Cattle ranching is the largest driver of deforestation in the Brazilian Amazon. The rainforest-to-pasture conversion affects the methane cycle in upland soils, changing it from sink to source of atmospheric methane. However, it remains unknown if management practices could reduce the impact of land-use on methane cycling. In this work, we evaluated how pasture management can regulate the soil methane cycle either by maintaining continuous grass coverage on pasture soils, or by liming the soil to amend acidity. Methane fluxes from forest and pasture soils were evaluated in moisture-controlled greenhouse experiments with and without grass cover (*Urochloa brizantha* cv. Marandu) or liming. In parallel, we assessed changes in the soil microbial community structure of both bare pasture soil as well as rhizosphere soil through high throughput sequencing of the 16S rRNA gene, and quantified the methane cycling microbiota by their respective marker genes related to methane generation (*mcrA*) or oxidation (*pmoA*). The experiments used soils from eastern and western Amazonia, and concurrent field studies allowed us to confirm greenhouse data. The presence of a grass cover not only increased methane uptake by up to 35% in pasture soils, but also reduced the abundance of the methane-producing community. In the grass rhizosphere this reduction was up to 10-fold. Methane-producing archaea belonged to the genera *Methanosarcina* sp., *Methanocella* sp., *Methanobacterium* sp., and Rice Cluster I. Further, we showed that liming compromised the capacity of forest and pasture soils to be a sink for methane, and instead converted formerly methane-consuming forest soils to become methane sources in only 40-80 days. Our results demonstrate that pasture management that maintains grass coverage can mitigate soil methane emissions, if compared to a bare pasture soil.

Keyword: climate change, soil methane cycling, environmental microbiology, soil conservation, microbiome management

52 Introduction

53 The establishment of pasture lands is the main cause of deforestation in the Amazon
54 region (Dias et al., 2016; Margulis, 2003). This transformation of rainforest into pastures leads
55 to the net increased emission of the powerful greenhouse gas methane, and turns a methane
56 consuming forest soil into a methane producing pasture soil (Meyer et al., 2020; Fernandes et
57 al., 2002; Steudler et al., 1996; Goreau; De Mello, 1988). The resulting greenhouse gas
58 emissions account for half of Brazil's greenhouse gas production, and have already exceeded
59 national emissions from fossil fuel by more than 20% (Bustamante et al., 2012; Fearnside &
60 Imbrozio Barbosa, 1998). Livestock production is responsible for the emission of several
61 greenhouse gases, and current studies work on minimizing this impact (Herrero et al. 2016,
62 Figueiredo et al. 2017). Recently, methane emissions from soil have become a focus of
63 investigations because they might be agriculturally manageable. The impact of land-use
64 conversion on the annual balance of gas fluxes is noticeable considering pastures in western
65 Amazonia can emit up to 270 mg C-CH₄/ m², while nearby forest soils can consume up to 470
66 mg C-CH₄/ m² (Steudler et al., 1996).

67 Methane (CH₄) gas has an 86-fold greater potential to retain heat in the atmosphere
68 compared to that of CO₂, calculated over a 20-year period (IPCC, 2013). The global methane
69 emissions are mainly driven by human activities such as livestock, irrigated agriculture, oil
70 and gas production, and landfill decomposition (IPCC, 2013). Soil methane cycling is strongly
71 dependent on the microbiota, since the biogenic source of this gas are methanogenic
72 archaea. The biological consumption of methane is controlled by methanotrophs, mostly
73 bacteria. In soil, the balance between methanotrophic bacteria and methanogenic archaea is
74 related to environmental conditions (i.e., moisture, temperature, soil density, and pH) and is
75 sensitive to changes in agricultural management (Le Mer & Roger, 2001; Liu et al., 2007; Tian
76 et al., 2015).

77 Methanotrophic bacteria in the soil are Gram-negative, belonging to
78 *Gammaproteobacteria* and *Alphaproteobacteria*, *Verrucomicrobia*, and candidates in the
79 phylum NC10 (Hanson & Hanson, 1996; Knief, 2015, Ettwig et al. 2010). The initial step of
80 methane oxidation occurs through its conversion to methanol, which is mediated by the
81 enzyme methane monooxygenase (MMO). Methanogenic archaea traditionally comprise
82 members from eight orders within the phylum *Euryarcheota*: *Methanopyrales*,

83 *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanocellales*,
 84 *Methanosarcinales*, *Methanomassiliicoccales*, and ‘*Candidatus* Methanophagales’ (Evans et
 85 al. 2019), with additional candidates in the phylum *Bathyarchaeota* (Kallistova et al., 2017).
 86 Methanogenesis is controlled by archaea and is the final step in an anaerobic pathway that
 87 begins with the hydrolysis of organic polymers, fermentation of the resulting monomers and of
 88 initial fermentation products, and ends up in the production of CH₄ mostly from acetate,
 89 hydrogen, and CO₂. The final step in the methanogenesis pathway is facilitated through the
 90 action of the enzyme methyl-coenzyme M reductase, coded by the *mcrA* gene, which can be
 91 used as a methanogen-specific marker for molecular studies (Serrano-Silva et al., 2014). The
 92 ability to produce methane has recently been demonstrated in cyanobacteria and plants,
 93 however, it is believed that this is a by-product from reactions of photosynthesis (Bižić et al.,
 94 2020; Keppler et al., 2006).

95 The relationship between changes in land-use and the response of the soil microbial
 96 community is not well understood (Nazaries et al., 2013; Tate, 2015), but previous studies
 97 have shown significant impacts on microbial diversity in the Amazon region (de Carvalho et
 98 al., 2016; Mendes et al., 2015; Navarrete et al., 2015; Rodrigues et al., 2013; Jesus et al.,
 99 2009). In well-managed pastures in the Amazon region, the grass root system can redistribute
 100 carbon to deeper layers, where it is less susceptible to decomposition (Fearnside & Imbrozio
 101 Barbosa, 1998). On the other hand, we can expect that degraded pastures with large bare
 102 soil areas can facilitate the release of carbon from the system, with superficial grassroots,
 103 higher loss of soil organic matter, and lower carbon stocks (Segnini et al. 2019). Proper
 104 management of pasture may involve several practices, such as soil acidity correction and
 105 continuous maintenance of grass cover to protect soil from erosion. These practices are
 106 particularly important in the Amazon region given the environmental extremes of this area.
 107 Altogether, the high soil acidity, high rainfall, and high temperatures combined with exposure
 108 of the soil to equatorial solar radiation constitute factors that are associated with increasing
 109 erosion and soil degradation (Demattê and Demattê, 1993). Soil degradation is the long-term
 110 decline in the soil’s productivity and its environment moderating capacity, with soil quality loss
 111 and reduction in attributes related to specific functions of value to humans (Lal, 2001).

112 The effect of soil liming on methane fluxes is still poorly understood, and studies of
 113 temperate forests show that liming can lead to both an increase and a decrease of methane

consumption (Wang et al. 2021; Borken and Brumme, 1997; Butterbach-Bahl et al., 2002). In wheat-focused agriculture liming has led to an increased consumption of methane in soil (Hütsch et al., 1994). The increased methane consumption after liming was also observed in Mediterranean semiarid soils under lupine, wheat, and triticale, a hybrid of wheat and rye (Barton et al., 2013; García-Marco et al., 2016). However, for tropical soils little information is available regarding what influence liming has on methane production and consumption. In an assessment of greenhouse gas fluxes from soils under soybean cultivation in Brazil, the acidity correction presented no effect on methane fluxes (Lammel et al., 2018). Likewise, in a field experiment in Puerto Rico, soil consumption of atmospheric CH₄ in an intentionally acidified soil was about one-fourth of that at pH 6, and was not restored after liming (Mosier et al., 1998).

There is a growing consensus that the key to understand major soil functions lies where plants and soil meet, in the rhizosphere (Lau et al., 2011). The rhizosphere is a micro-environment with differentiated soil conditions and steep overlapping gradients, in which pH can be up to 2 units more acidic or more basic than the soil surrounding the rhizosphere. The rhizosphere can present heterogeneous concentrations of oxygen and moisture and can be enriched in root exudates like sugars and organic acids (Philippot et al., 2013). These factors affect soil methane cycling not only by providing organic substrate for methanogenesis but also by promoting the oxidation of methane in the rhizosphere. Despite its known role in flooded rice soils (Frenzel et al., 1992), little is known about the impact of the rhizosphere on methane cycling in upland soils since these soils are commonly considered to be a methane sink, not a source (Philippot et al., 2009). Thus, improved understanding of how the rhizosphere of land-intensive tropical pastures affects soil methane cycling can yield new strategies to mitigate greenhouse gas emissions related to cattle ranching.

The intensive land use in agriculture and cattle ranching in Amazonia leads to soil and pasture degradation ranging from 50% to 70% of total area (Dias-Filho, 2017). Within this context this research aims to evaluate how the management of pastures can affect soil methane cycling. We hypothesized that CH₄ production is reduced by liming soils and by continuous grass coverage due to the influence of the rhizosphere of *Urochloa brizantha* cv. Marandu, a grass widely used in pastures in Brazil. To test this hypothesis, we measured methane fluxes in soils from pasture field sites with and without grass cover, and compared

the microbiota in bare pasture soil to that of the rhizosphere of grass covered soil. These studies were complemented with greenhouse experiments where soil acidity was adjusted and grass was planted, gas flux rates of the soil-air CH₄-fluxes were measured, and shifts in the soil microbial community between bare soil and the rhizosphere of *Urochloa brizantha* cv. Marandu were determined.

Materials and Methods

Sampling

These studies were performed with soils from both western and eastern Amazonia. In the western region (hereafter “Ariquemes”) sampling was carried out in April of 2017 at the Fazenda Nova Vida near Ariquemes, RO (10°10'49.5" S, 62°49'23.9" W). While in the eastern region (hereafter “Tapajós”), the samples were taken at the National Forest of Tapajós and the immediately surrounding areas near Belterra, PA (3°07'53.8" S, 54°57'24.2" W), in August of 2019. The sampled soils were used in two rounds of greenhouse experiments at the Center for Nuclear Energy in Agriculture, SP, Brazil (22°42'27.7" S, 47°38'41.0" W). In addition to the soil sampling a field study was performed, but only in the Tapajós region (detailed below).

Western Amazonia has been studied for the impacts of conversion from forest to pasture, with extensive scientific literature characterizing ecosystem responses to conversion (de Moraes et al., 1996; Herpin et al., 2002; Reiners et al., 1994) for representing a region with a high degree of exploitation. The Fazenda Nova Vida region has fragments of primary forest and pastures of different ages. The sampled pasture area was established in 1972, and since then managed by cattle rotation, with the use of fire only to control eventual pests, mechanical removal of invasive trees, and at least one record of liming 15 years before the sampling. Soils sampled varied from average clay to sandy texture.

Eastern Amazonia represents areas of more recent exploration. The Tapajós National Forest was sampled as a model of a conservation area and the pasture chosen is in a small property in Belterra, PA. The pasture used here was established between 1989-1994 and supported cattle at the time of sampling, had sparse signs of degradation, fire was applied when necessary to control invasive plants, and it has no history of liming. Soils sampled varied from average clay to sandy texture.

During each expedition, we sampled 20-30 kg of soil from the upper 0-10 cm layer of 5 equidistant sampling points along a linear gradient of 200 m at each site, from areas under Primary Forest and Pastures with *Urochloa brizantha* cv. Marandu. The sampled soils were transported to the Cell and Molecular Biology Laboratory at the University of Sao Paulo, CENA-USP, with fresh samples for chemical analysis and greenhouse experiments, or were frozen at the end of each sampling day and stored at -20 °C for future molecular analyses.

Field Study

At two pastures in the Tapajós region, 100 m side squares were established and 4 points in the square corners, plus a point in the center, were selected to evaluate CH₄ fluxes and to sample soils for molecular and chemical analysis. Those 5 points had grass coverage at the time, and prior to gas flux measurements with static gas collection chambers the grass leaves were cut to their stems (2 cm above soil surface) and removed. Following chamber removal, the roots were collected, and the rhizospheric soil was sampled and stored at -20 °C. Adjacent to each of the five sampling points we had selected, one square meter large areas without grass (bare soil) we used to measure methane fluxes and to collect soil samples for molecular and chemical analysis.

Greenhouse experiments

Sampled soil was homogenized, sieved (5 mm), and placed in clay pots with a capacity of 10 liters, resulting in 10 cm high soil columns with 5 kg of soil per pot. The grass was raised from seed in a subsample of the soil, and mature plants were transferred to the experimental clay pots at least 40 days after soil liming. The liming was performed by the addition of CaCO₃ to reach pH 6.5 (water), calculated for a base saturation of 70-75%. For each treatment, four pots were used to grow *Urochloa brizantha* cv. Marandu with 4 additional pots as no-plant controls (bare soil), both at natural pH and with limed soils (4 pots x 2 soil types x 2 pH situations x 2 plant situations). At the beginning of the experiment soil moisture was standardized to ~70% of the water retention capacity of the soil and adjusted every two to four days, taking as reference the weight variation after drying soil samples for 48 h at 75 °C. In the experiment with soil from Ariquemes, the plants were removed when they reached approximately 35 cm in height, and by shaking and with the help of a sterilized brush the

rhizosphere soil was collected. Here we defined the rhizosphere as soil that remained attached to the roots even after vigorous plant shaking.

Determination of methane fluxes in soil

The measurements of CH₄ fluxes in both the field sampling sites and the greenhouse experiments were carried out using static gas collection chambers (20 cm in diameter, ~6 L inner volume). Over a period of 10 minutes measurements were taken at 10 second intervals using a portable gas analyzer (UGGA, Los Gatos Research, San Jose, CA, USA). Daily flux of gases was estimated from the concentration in the chamber headspace. Daily flux (F, mass of gas m⁻².day⁻¹) was computed as (Ussiri, Lal and Jarecki, 2009):

$$F = \Delta \text{gas} / \Delta t \times V / A \times k$$

Where $\Delta \text{gas} / \Delta t$ is the rate of change in CH₄ concentration inside the chamber (i.e. mg CH₄-C); V is the chamber volume (m³); A is the surface area circumscribed by the chamber (m²) and k is the time conversion factor (1440 min day⁻¹). The cumulative gas emissions were calculated by linear interpolation of average emissions between two successive measurements and the sum of the results obtained over the entire study period. Finally, the data was expressed as differences in the cumulative CH₄ fluxes in relationship to the controls. From this, we subtracted from the accumulated fluxes in the respective treatments (liming, grass coverage, and liming plus grass coverage) the control measurements of the average accumulated flux (bare soils).

Characterization of soil chemical properties

About 600 g of soil were analyzed for their physical-chemical properties at the Laboratory of Chemical Analysis in the Soil Science Department at the “Luiz de Queiroz School of Agriculture” (ESALQ / USP) (detailed in van Raij et al., 2001). The soil attributes measured were: pH in CaCl₂; concentrations of phosphorus, potassium, calcium, and magnesium by extraction with ion exchange resin; aluminum by extraction of potassium chloride at 1 mol/ L; potential acidity estimated by pH- SMP buffer test; organic matter by the dichromate- titrimetric method; boron by extraction with hot water; copper, iron, manganese

and zinc extracted by the DTPA-TEA extractor (pH 7.3); and by calculating the sum of bases (BS); cation exchange capacity (CEC); base saturation (V%), and aluminum saturation (m%).

DNA extraction

DNA was extracted for molecular analyses from greenhouse soils that originated in Ariqueles and Belterra (Tapajós region) and from soils of the field study in the Tapajós regions. Total DNA was extracted from soil samples using the PowerLyzer PowerSoil DNA Isolation Kit (Qiagen, Hilden, Germany) from 250 mg of soil, according to the protocol provided by the manufacturer, except that after adding solution C1 the stirring time was extended to 15 minutes followed by 3 min centrifugation (Venturini et al., 2020). The amount and quality of the DNA extracted was analyzed in a Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) at an optical density of 260 nm. The total DNA extracted was stored at -20 °C.

Abundance of methane producers and oxidizers

Real-time quantitative PCR (qPCR) was used to quantify the genes associated with methane cycling *mcrA* and *pmoA* (Table S1) in total soil DNA samples. For each gene, a standard curve was established spanning each order of magnitude from 10^1 to 10^7 copies of the gene. Target genes were previously obtained by PCR from genomic DNA of *Methanolinea mesofila* (DSMZ 23604) for the *mcrA* gene, and *Methylosinus sporium* (DSMZ 17706) for the gene *pmoA*, both obtained from the DSMZ (German Collection of Microorganisms and Cell Cultures). The qPCR was performed in triplicate for each sample on a StepOne Plus cycler (Thermo Fisher Scientific, Waltham, MA, USA), with a final volume of 10 µL, containing 5 µL of SYBR Green ROX qPCR (Thermo Fisher Scientific, MA, USA), 1 µL of each primer (5 pmols), 1 µL of soil DNA (adjusted to 10 ng/ µL), 0.8 µL of bovine albumin (20 mg / mL) (Sigma-Aldrich, San Luis, MO, USA), and 1.2 µL of ultrapure water (Milli-Q, autoclaved).

In order to minimize bias in the analysis between each qPCR plate run, gene abundance was quantified with the software LinRegPCR (Ramakers et al., 2003). Raw amplification data for each sample were used to calculate individual reaction efficiencies, and detection limits were established for each group of technical replicates. The data generated in arbitrary fluorescence units were converted to the number of copies of the genes using linear

interpolation between the known quantities in the standard curve (5 best points out of 7) and the observed fluorescence measurements, using the curves of each plate as a reference for the respective samples.

Sequencing of 16S rRNA gene fragments

The composition of the microbial community was determined with high throughput sequencing (MiSeq Illumina platform with a 600c kit) of the V4 region of the 16S rRNA gene at the Functional Genomics Center of Luiz de Queiroz College of Agriculture (Caporaso et al., 2011). The V4 region was amplified with the primers 515F (Parada et al., 2016) and 806R (Apprill et al., 2015). This sequencing strategy was selected to match the highly diverse soil environment and for the size of the paired-end reads (average 300 bp). Gene library preparation followed the conditions of 95°C for 3 minutes, followed by 25 cycles at 95°C for 30 seconds, 50°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for 5 minutes. The DNA concentrations in the samples were adjusted to 10 ng/μL using a Nanodrop 2000c spectrophotometer and the PCR reactions with 2.5 μL of 10x buffer, 1 μL of 50 mM MgCl₂, 1 μL of 10mM dNTPs, 0.5 μL of 10μM Forward and Reverse Primers, 0.5 μL of 5 U/ μL Taq Platinum - PCR and water for PCR - 14 μL, in a total volume of 25 μL per reaction. Subsequent DNA purification of the amplicon was performed using AMPure XP beads (Beckman Coulter, Brea, CA, USA) and verified on an agarose gel. In a similarly structured, second PCR the adapters were added, followed by another purification with AMPure XP beads and gel electrophoretic confirmation in agarose. The amplicon pool was normalized using quantification by qPCR with the KAPA Illumina quantification kit (Roche, Basel, Switzerland). The computational processing of these data was performed using QIIME2 2017.11 (Bolyen et al., 2019), with data quality control using the DADA2 tool (Callahan et al., 2017), without clustering into OTUs, and taxonomic identification of the sequences was performed using q2-feature-classifier (Bokulich et al., 2018) and the SILVA v.128 99% database (Quast et al., 2013).

300 **Phylogenetic analyses**

301 Some amplification sequence variants (ASVs) grouped closest with the family
302 Beijerinckiaceae. Since this family represents both methanotrophic and non-methanotrophic
303 genera, we increased our phylogenetic resolution by analyzing phylogenetic trees containing
304 only Beijerinckiaceae sequences that were created with two 16S rRNA primer pairs. One set
305 of primers targeted the region V4, between 515F (Parada et al. 2006) and 806R (Apprill et al.
306 2015), and a second set of primers targeted the V3/V4 region, between 341F and 805R
307 (Herlemann et al., 2011). For this last pair, the amplification protocol was identical to that
308 described above, except the annealing temperature for the second primer pair was 55°C. The
309 sequences were aligned, and trees were calculated using the software CLC Genomics
310 Workbench 20.0 (QIAGEN, Aarhus, Denmark) at default parameters, and with a maximum
311 likelihood model (PHYML function) with UPGMA (Unweighted Pair Group Method with
312 Arithmetic mean) assuming common replacement frequencies to the bases (Kimura, 1980).
313 The robustness of the final trees was tested with 1000 bootstrap replications. Reference
314 sequences for members of the Beijerinckiaceae family were obtained from the cured
315 database RDP version 11 (Cole et al., 2014) based on the criteria of high-quality reads with a
316 length greater than 1200 bp and representing type strains. The only sequence available for
317 the 16S rRNA gene of the methanotrophic bacterium USCα (Pratscher et al., 2018) was also
318 added.

319

320 **Statistical analysis**

321 All comparative analyses between groups were performed with ANOVA followed by a
322 Tukey Honestly Significant Difference (HSD) test and p-values calculated for a two-tailed
323 distribution of the data using the package *agricolae* version 1.2-8 (R Core Team, 2013).

324 Significant explanatory variables of the methane fluxes were chosen by linear
325 regression and model selection (backward) and by minimizing the Akaike Information
326 Criterion (AIC). The statistical significance was assessed by 1000 permutations of the
327 reduced model. The resulting significant explanatory variables were used to access their
328 contribution to explain the CH₄ fluxes, using the function *varpart* (Peres-Neto et al., 2016) in
329 the *vegan* package (Oksanen et al., 2015). Statistical analyses were performed in R Studio
330 software (R Core Team, 2013).

The DEICODE tool (Martino et al., 2019) in the QIIME2 2019.10 was used to process the sequencing data. This tool can identify significant changes in the community based on relative abundance data. Next, the software QURRO (Fedarko et al., 2019) was used to assess shifts in the methane cycling community based on transformed abundance data (natural logarithm) and using a minimum of 10 occurrences per taxon.

Results

This study tested the effect of acidity correction by liming and the presence of a grass cover by *Urochloa brizantha* cv. Marandu on soil methane fluxes with soils from pasture and forest of different Amazon regions. Two greenhouse experiments were set up. The first experiment was performed with soils from a western Amazon region (Ariquemes, RO) and the second with soils from an eastern region (Tapajós, PA). In both experiments liming resulted in a final pH of ~6.0 (CaCl₂, equivalent to pH 6.5 in H₂O), and an increase in calcium availability, as well a decrease in aluminum saturation (Table S2). In the Ariquemes experiment, methane was sink in both bare soils of forest and pasture at their respective natural pH values, with greater uptake in forest soils (Figure 1). In the Tapajós experiment, we observed methane emissions from bare soils from the pasture at natural pH, and methane uptake in bare soils from forest at natural pH (Figure S1). When forest soils from Ariquemes had grass cover they exhibited the highest methane consumption (Figure 1-b; $p = 0.059$), at values close to the naturally acidic forest soil, but significantly lower than both limed soils with or without grass cover (Figure 1-b). The Tapajós soils showed a similar trend compared to Ariquemes soils (Figure S1). Methane uptake in pasture soils increase by 35% on average when they have grass coverage (Figure 1-a; $p = 0.001$). However, liming of pasture soils reduced their methane uptake (Figure 1-a; $p = 0.001$) and turned forest soils from a methane sink into a methane source (Figure S1-b; $p = 0.052$).

357 Figure 1: Differences in the accumulated methane flux effect compared to untreated control (bare
358 soils at natural pH) in a) pasture and b) forest soils from the Ariquemes experiment in western
359 Amazonia, with and without acidity correction and with and without grass coverage. Bars show
360 standard deviation.
361

362 The validity of our observation that, compared to exposed bare soils, grass coverage
363 improves soil methane uptake was tested in the field. *In situ* measurements of CH₄ fluxes
364 were taken on two pastures in Belterra/PA, Tapajós region, during the end of wet season, at
365 points with and without grass coverage. No significant differences were observed, but the
366 trend is similar to that observed in the greenhouse experiments (Figure S2; $p = 0.112$).

367 Molecular analyses were performed only with soils from the Ariquemes greenhouse
368 experiment (Figure 2) and from the field study in the Tapajós region (Figure S3). The
369 methane cycling microbiota was evaluated through quantification of their functional marker
370 genes *pmoA* and *mcrA*, indicating methane consumers and producers, respectively (Figure
371 2). The rhizospheric community was evaluated only at the end of the experiment (T3=250
372 days). During most of the experimental timeline, we did not observe differences in the
373 abundance of methanotrophs between pasture and forest soils (Figure 2). Regarding
374 methane producers, we observed very low abundance in forest soils compared to pasture
375 soils during the experimental duration. The acidity correction shows a tendency to reduce
376 methanotroph levels in forest soils after 250 days in the grass rhizosphere ($p = 0.339$) and in
377 the bare soil ($p = 0.162$) (Figure 2A). Pasture soils had between 100 and 1000-fold more
378 methanogenic archaea than forest soils throughout the experiment, which did not change with
379 acidity correction (Figure 2B). The abundance of methanogenic archaea in the grass
380 rhizosphere in pasture soils was reduced on average by 13 times compared to the bare soil

381 (Figure 2B; $p = 0.025$). No significant changes of methanotrophs were recorded in the
 382 rhizosphere (Figure 2A; $p = 0.263$). This reduction in methanogenic archaea in the grass
 383 rhizosphere was not observed in the field study (Figure S3; $p = 0.186$).
 384

385 Figure 2: Gene quantification of the genes (A) *pmoA* and (B) *mcrA* at different times after the
 386 beginning of the Ariqueles experiment. T1 = 40 days, T2 = 130 days and T3 = 250 days. Letters
 387 above each box plot indicate significant changes ($p < 0.05$). CN = copy number. Ns = Not significant.
 388

389 To investigate the effects of acidity correction and grass rhizosphere on specific groups
 390 of microorganisms, high throughput DNA sequencing of the 16S rRNA gene was performed.
 391 The results show fair sequencing depth, with rarefaction curves leveling off well below the
 392 minimal sequencing depth in soils from the Ariqueles experiment (Table S3, Figure S4-a)
 393 and in soils from the field studies (Table S4, Figure S4-b).

394 Considering only the community associated with methane cycling in the soil (identified
 395 with a minimum of 90% confidence), we filtered the groups recognized as methanotrophs
 396 (Knief, 2015) and methanogens (Evans et al., 2019) and observed results similar to those
 397 obtained in the quantification of gene copies in total DNA. The number of methanogens in
 398 forest soils was smaller, while the abundance of methanotrophs was similar between forest

399 and pasture soils (Figure 3). The increase of methanogens in forest soils with acidity
 400 correction was not significant. A significant drop in methanotroph abundance was observed
 401 only for the combination of acidity correction and grass cover treatments (Figure 3, $p=0.024$).
 402 Methanotrophs in pasture soils did not change with liming or with the presence of grass cover,
 403 as previously observed in the quantification of *pmoA* gene copies (Figure 2). However, the
 404 number of methanogens was significantly reduced in the grass rhizosphere, with ($p=0.017$) or
 405 without ($p=0.007$) acidity correction (Figure 3). This last result was similar to that observed in
 406 the field, which, although not significant, points to a tendency to reduce methanogenic
 407 archaea of different groups in the grass rhizosphere (Figure S5).
 408

410 Figure 3: Changes in the logarithmic ratio between methanogenic and methanotrophic groups in
 411 relation to the whole community (16S rRNA) in the Ariqueles experiment. The dotted line indicates a
 412 calculated ratio of the minimum of 10 counts for each group. The more negative the number of the
 413 natural log, the lower the abundance in relation to the total community. Letters indicate significant
 414 changes within each treatment for the same land use and group of microorganisms (Tukey HSD; $p <$
 415 0.05). Ns = not significant.
 416

417 Finally, to understand which groups are associated with methane cycling in these soils,
 418 a detailed analysis was performed of all groups that presented sequences of the genera
 419 known to act in methane cycling (Knief, 2015; Angel et al., 2012) (Figures 4 and 5). In pasture
 420 soils, the abundance of members of all methanogenic genera was lower when soil was grass
 421 covered. Forest soils showed a low abundance of methanogenic archaea belonging to
 422 *Methanosarcina* spp. The Beijerinckiaceae family is abundant in these soils but it was not
 423 possible to identify the sequences at the genus level with the database SILVA v.128 (Quast et
 424 al., 2012). A new phylogenetic identification was performed comparing all the sequences
 425 annotated as Beijerinckiaceae in the RDP database, with the five amplification sequence
 426 variants (ASVs) from sequencing with primers 341F/805R and 8 ASVs from primers
 427 515F/806R. In this analysis, only sequences of the 16S rRNA gene of this family were used
 428 as reference, and the results indicate that they are closely related to the methanotrophic
 429 clade, with more than 90% confidence (Figures S6 and S7). Those Beijerinckiaceae are
 430 reduced in their relative abundance in forest soils with acidity correction (Figure 5), without
 431 changes in pasture. No significant changes were observed in relation to other methanotrophs.
 432
 433

Figure 4: Changes in the Log_e ratio between methanogenic microorganisms by genus (G) in relation to the total community in the Ariqueles experiment. All the identified genera are shown. The more negative the numbers, the lower the abundance. Letters indicate significant differences (Tukey HSD; p<0.05)

439

440

Figure 5: Changes in the Log_e ratio between methanotrophic microorganisms by genus (G) or family (Fa) in relation to the total community in the Ariqueles experiment. The more negative the numbers, the lower the abundance. Letters indicate significant differences (Tukey HSD; p<0.05). Analysis showed that a large part of the explained variation (67%) was due to the joint affects.

To disentangle the effect of soil properties, microbial communities, and the presence of grass on the CH₄ fluxes we performed a variation partitioning analysis. This analysis showed that a large part of the explained variation of the methane fluxes (40%) was due to pH and other soil properties in the first days of the experiment (Figure 6b). However, the grass biomass represented most of the explained variance at the later time-points (Figure 6e-f). The effect of methanogen and methanotroph abundance was minor in the beginning of the incubation (6%) but reached 13% of explained variance at the last time point. These results

457 indicate that the CH₄ fluxes vary through time and are driven by dynamic factors. Here we
 458 decided to separate pH from other soil properties when running this analysis as pH has been
 459 previously shown to be a major driver in CH₄ soil fluxes. Our results confirm this as in the
 460 beginning of the experiment the pH and other soil physical-chemical properties were the
 461 stronger explanatory variables of the CH₄ uptake capacity (Table 1). However, the
 462 contribution of the soil properties decreases through time while the presence of the grass and
 463 the microbial communities gain in explanatory power.
 464

Figure 6: Variation partitioning analysis to determine the drivers of CH₄ fluxes in the Ariqueemes soils in time intervals from day 0 to day 7 (b), day 18 to 28 (c), 28-84 (d), 84-96 (e), and 96-1-8 (f). Variance was partitioned into four explanatory variables, soil physical-chemical properties (S), pH, abundance of methanotrophs and methanogens (G), plant biomass (P), and by combinations of these potential predictors as exemplified in the outline (a). Geometric areas are proportional to the respective percentages of explained variation. The corners of the square depict the variation explained by each factor alone, while percentages of variation explained by interactions of two or all factors are indicated on the sides and in the middle of the square, respectively. All numbers represent percentages, graphically represented by the size of the respective hexagons. Only variance fractions $\geq 2\%$ are shown. The variables used for each variation partitioning are indicated in Table 1.

Table 1. Selected explaining variables for the capacity of methane consumption determined with the variation partitioning analyses at five different time-intervals during the Ariqueemes experiment.

Time interval	Soil properties ^a & pH	Gene abundances	Plant ^b
0-7	pH, OM, Cu, CEC	<i>mrcA</i>	NA
18-28	pH, Cu	<i>pmoA</i>	NA
28-84	pH, CEC	-	NA
84-96	-	-	Plant biomass
96-108	-	<i>mcrA</i>	Plant biomass

^aCEC corresponds to Cation Exchange Capacity, Cu to Copper, and OM to Organic Matter.

^bPlant biomass corresponds to the sum of the above and belowground dry weight.

486 Discussion

487 Deforestation of the Amazonian forest often followed by the establishment of pastures.
 488 This forest-to-pasture conversion affects soil methane cycling, where forest soils that were
 489 previously acting as a methane sink now become sources of methane (Fernandes et al.,
 490 2002). This study confirms previous field results (Meyer et al., 2020; Fernandes et al., 2002;
 491 Goreau; De Mello, 1988; Steudler et al., 1996), although the values obtained in our
 492 experiments cannot be directly compared to those reported, since the results are limited to a
 493 10 cm surface layer of soil. The trend observed is the same recorded by Steudler et al.
 494 (1996), in that forests consume 2.74 more methane than pastures. In our experiments, these
 495 values were 0.6-fold in soils from Ariquemes in western Amazonia, and 4.28-fold in soils from
 496 Tapajós in eastern Amazonia. This discrepancy may be related to differences in soil
 497 microbial communities and chemical properties, but it can also be a consequence of
 498 conservation of the forest areas from which these soils originated. While in Ariquemes forests
 499 were fragmented and small samples from Tapajós originated from a contiguous forest in a
 500 conservation area. Forest fragmentation is known to be associated with increased
 501 greenhouse gas emissions (Laurance et al., 1998). Furthermore, the pastures sampled in
 502 Ariquemes/RO have a history of long-term management, and the pasture soils sampled in
 503 Belterra/PA showed signs of degradation. As management affects the carbon stock in the soil
 504 (Fearnside & Imbrozio Barbosa, 1998), it can be expected that it will also affect methane
 505 cycling in the soil.

506 The forest-to-pasture conversion alters the physical-chemical properties of the soil,
 507 with consequences to microorganisms that produce and consume methane. In field studies in
 508 the same region in Ariquemes, a decrease in methanotrophic bacteria and an increase in
 509 methanogenic archaea were observed, in addition to changes in the composition of
 510 communities, which were attributed at least in part to changes in methane fluxes (Meyer et
 511 al., 2017, Meyer et al. 2020). Also, an increase in activity of methanogens in pastures,
 512 compared to forest soils, was recorded in soils from the same region (Kroeger et al. 2020).
 513 Our results under controlled moisture conditions did not detect significant changes in the
 514 methanotrophic community nor changes in the relative abundance of specific methanotrophic
 515 groups. However, there was a significant increase in the abundance of methanogenic
 516 archaea in pastures compared to forest soils. These results indicate that studies of the

microbiota associated with methane cycling should consider the seasonality of rainfall in the field to better understand the system, such as that developed by Fernandes et al. (2002).

Pasture soils in the Amazonian region present a microbial community quite distinct from that observed in forest soils (de Carvalho et al., 2016; Rodrigues et al., 2013; Jesus et al., 2009). This is partly attributed to acidity reduction in the process of establishing pastures. Forest soils in Amazonia have pH (H₂O) values between 3.5 to 4.5 (Demattê and Demattê, 1993). The pH is currently understood as one of the main drivers of microbial community structure in soils (Fierer & Jackson, 2006), and increasing it by liming is a strategy to improve fertility and reduce soil toxicity to plants (Oliveira et al., 2013). This process becomes necessary in pasture management to counter the tendency for acidification of pasture soils over time with soil pH reaching values close to those observed in forest areas (de Moraes et al., 1996). In addition, degraded pastures, which can amount to more than 50% of pasture areas in Amazonia (Dias-Filho, 2017), also tend to need acidity correction for their restoration.

Little is known about the effects of soil liming on the methane cycling process in tropical soils. It is known that the optimum growth pH of most cultivable methanotrophs and methanogens is neutral (Le Mer & Roger, 2001; Whittenbury et al., 1970), which is why soil pH represents an important explanatory variable for the distribution of methanotrophs. However, methane oxidation is observed in natural environments across a wide pH range (Knief et al., 2003; Kolb, 2009; Nazaries et al., 2013). Our results indicate that the soil acidity correction for pH (H₂O) values close to 6.5 has different effects in pasture or forest soils, possibly because the forest soil has undergone a more intense pH correction, starting at 3.5-5.0 and finishing at 6.5, while in pasture soils the change was from 4.5-5.5 to 6.5. In our forest soils from Ariquemes, we determined a decrease in methane uptake in response to liming, and a shift from uptake to emission in forest soils from Tapajos. Yet, no significant differences were noticed in pasture soils. Thus, liming pasture soils may not impact methane emissions, but still help to maintain the pH of these soils at values suitable for grass biomass productivity. For forest soils, we have shown that the reduction in acidity alone is enough to shift the soil from a methane sink to a source. This change in methane fluxes was not noticeable in the abundance of methanotrophic or methanogenic microorganisms by qPCR, despite a reduction in the relative abundance of methanotrophs that follows the acidity correction.

548 The identification of microorganisms based on short DNA sequences of the 16S rRNA
 549 gene, such as those generated in this study, is limited to the evolutionary information
 550 available in that fragment so that it is not always feasible to identify the microorganisms at the
 551 genus level. Considering that the ability to oxidize methane is variable at the genus level in
 552 the Beijerinckiaceae family, identifying sequences at the family level is not enough to infer if
 553 they are methanotrophs. This family also includes generalist bacteria capable of using
 554 multiple carbon compounds as an energy source, and here Beijerinckiaceae are more
 555 abundant in forest soils than in pastures. Thus, identifying whether they are methanotrophs or
 556 not is relevant to understand methane cycling in the forest-to-pasture conversion. The results
 557 demonstrate that the Beijerinckiaceae sequences observed in forest soils cluster together in
 558 phylogenetic trees. This cluster was observed on the two data sets with high support (> 90%
 559 in 1000 bootstraps) and also includes the methanotrophic USC α , which indicates that these
 560 sequences are potential methanotrophic Beijerinckiaceae.

561 The differences observed in methane fluxes after liming were not noticed in the
 562 abundance of producers and consumers. This discrepancy might be related to a reduction in
 563 the activity of forest soil to act as a methane sink after acidity correction, due to the lower
 564 availability of Fe and Cu which are necessary as cofactors for the activity of methane-
 565 monooxygenase (Semrau et al., 1995). Alternatively, this discrepancy might be due to
 566 limitations of the primers, drawn mostly with microbial references from temperate soils, but we
 567 applied them to tropical soils. Or, the difference between methane flux and shift in abundance
 568 of methane cycling microorganisms can be due to ammonia oxidizers, possibly oxidizing
 569 methane at a higher soil pH. We believe that methanotrophs are the group that was affected
 570 the most, since acidity correction was followed by a reduction in the consumption of
 571 atmospheric methane by the soil (concentrations of ~1.8 ppm). Also, the duration of
 572 incubating soil from Ariqueles for 250 days should be long enough to observe compensatory
 573 changes due to DNA replication, that should be detected in the DNA quantification analysis.

574 Although there is great natural variability in the methane flux data, the final averages
 575 led to the conclusion that pasture soils act as a methane source, which is in fact a commonly
 576 reported final result. This observed variability means that pastures could seasonally or by
 577 location switch from being a methane source to temporarily becoming a methane sink
 578 (Fernandes et al., 2002; Steudler et al., 1996). Our initial hypothesis was that the methane

579 consuming capacity of pasture soils would be related to intermediate moisture availability in
 580 the micro-environments of soil, since soil moisture is a determining factor for methane fluxes
 581 in pastures (Verchot et al., 2000). To eliminate moisture variation as a variable in the
 582 experiments we set the soil water contents at 70% of the holding capacity in the greenhouse
 583 experiments. The variability of pasture gas fluxes could also be explained by grass coverage,
 584 a factor associated with pasture management. The management of pastures can influence
 585 soil gas fluxes (Figueiredo et al. 2017), since it influences the carbon stocks in the soil
 586 (Fearnside & Imbrozio Barbosa, 1998), however the way ongoing pasture management can
 587 affect the microbial community remains an open question. Considering that management is
 588 performed with the goal of grass productivity, and greater aerial biomass is associated with
 589 greater root biomass, we expect that a larger root surface area in pasture would create a
 590 more interactive environment with the soil microbiota, and thus enable higher rhizosphere
 591 activity. The role of the rhizosphere on methane cycling in upland soils is still poorly
 592 understood, and even different plant species can influence the soil by increasing methane
 593 oxidation or production, depending on the type of soil or soil conditions (Praeg et al., 2017). In
 594 soils of the Ariqueles experiment, we observed that plant cover will lead to a reduced
 595 methane flux in both forest and pasture soils compared to those with acidity correction. The
 596 methane flux rates with grass cover were similar to those of the original forest soil and tended
 597 to be higher than those of pasture without acidity correction. In soils from Tapajós experiment,
 598 the same trend was observed in forest soils, but possible due to the shorter duration of this
 599 experiment, there were no significant differences in the pasture.

600 When disentangling the contribution of different biotic and abiotic factors to CH₄ soil
 601 uptake capacity we found that its drivers change through time, which could explain as
 602 previously discussed that soils might change from a source to a sink. While pH and other soil
 603 properties explained most of the variance in the beginning of the greenhouse experiment, the
 604 abundance of microbial communities related to CH₄ fluxes and plant biomass explained most
 605 of the CH₄ uptake at the end of the experiment. These results suggests that our treatments
 606 (liming and planting grass) are causing a reorganization of the microbial communities and
 607 while the soil properties are initially the main variables explaining the CH₄ fluxes, after a
 608 couple of weeks the biotic factors are the main drivers of CH₄ fluxes in these soils. While a
 609 previous study showed that peak emissions of the green-house gas N₂O can be driven by the

microorganisms related to the production and reduction of this green-house gas (Domeignoz-Horta, et al., 2017), our results show how microorganisms related to methane cycling and plant cover play a role to understand the temporal dynamics of CH₄ uptake in soils. These results highlight the need for better characterizing microbial communities to increase our understanding of the relationship between abundance and diversity of microorganisms and their corresponding processes.

The results presented here demonstrate that soil acidity is an important factor for methane sequestration in tropical soils, as the acidity correction reduces this capacity. In pastures, the effect of the acidity correction is less consequential compared to the presence of grass coverage. This demonstrates that the correction of acidity in pastures, if combined with constant soil coverage with grass, would have little or no impact on methane emissions while improving soil structure and increasing nutrient availability, soil organic matter and grass productivity.

Conclusion

Our results suggest that the impact of methane emissions from pastures in the Amazonian region can be mitigated through pasture management, specifically by keeping the soil always covered with grass. The rhizosphere of *Urochloa brizantha* cv. Marandu affects soil microbial communities by lowering the abundance of methanogenic archaea up to 10 times compared to the bare soil. The affected methanogens are composed of *Methanobacterium* spp., *Methanocella* spp., *Rice Cluster I*, and *Methanosarcina* spp. In addition, we demonstrate that the correction of acidity in pasture soils can reduce methane sequestration under atmospheric methane concentrations (high-affinity methanotrophs). Therefore, the level of acidity correction should be considered as a factor for additional emissions of greenhouse gases. In the acidic forest soils, an increase in pH reduced methane sequestration by more than 50%, thereby reversing the flux direction to turn forest soil from a methane sink into a source. Field studies with liming and a focus on the grass rhizosphere under seasonal conditions are urgently needed to provide specific recommendations to policymakers and farmers.

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655 References

- 656 Angel, R., Claus, P., & Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in
657 aerated soils and become active under wet anoxic conditions. *The ISME Journal*, 6(4),
658 847–862. <https://doi.org/10.1038/ismej.2011.141>
- 659 Apprill, A., McNally, S., Parsons, R., & Weber, L. (2015). Minor revision to V4 region SSU
660 rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. *Aquatic*
661 *Microbial Ecology*, 75(2), 129–137. <https://doi.org/10.3354/ame01753>
- 662 Barton, L., Murphy, D. V., & Butterbach-Bahl, K. (2013). Influence of crop rotation and liming
663 on greenhouse gas emissions from a semi-arid soil. *Agriculture, Ecosystems &*
664 *Environment*, 167, 23–32. <https://doi.org/10.1016/j.agee.2013.01.003>
- 665 Bižić, M., Klintzsch, T., Ionescu, D., Hindiye, M. Y., Günthel, M., Muro-Pastor, A. M., Eckert,
666 W., Urich, T., Keppler, F., & Grossart, H.-P. (2020). Aquatic and terrestrial cyanobacteria
667 produce methane. *Science Advances*, 6(3), eaax5343.
668 <https://doi.org/10.1126/sciadv.aax5343>
- 669 Bokulich, N. A., Kaehler, B. D., Rideout, J. R., Dillon, M., Bolyen, E., Knight, R., Huttley, G. A.,
670 & Gregory Caporaso, J. (2018). Optimizing taxonomic classification of marker-gene
671 amplicon sequences with QIIME 2's q2-feature-classifier plugin. *Microbiome*, 6(1). <https://doi.org/10.1186/s40168-018-0470-z>
- 673 Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A.,
674 Alexander, H., Alm, E. J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J. E., Bittinger, K.,
675 Brejnrod, A., Brislawn, C. J., Brown, C. T., Callahan, B. J., Caraballo-Rodríguez, A. M.,
676 Chase, J., ... Caporaso, J. G. (2019). Reproducible, interactive, scalable and extensible
677 microbiome data science using QIIME 2. In *Nature Biotechnology* (Vol. 37, Issue 8, pp.
678 852–857). Nature Publishing Group. <https://doi.org/10.1038/s41587-019-0209-9>
- 679 Borken, W., & Brumme, R. (1997). Liming practice in temperate forest ecosystems and the
680 effects on CO₂, N₂O and CH₄ fluxes. *Soil Use and Management*, 13, 251–257.
681 <https://doi.org/10.1111/j.1475-2743.1997.tb00596.x>
- 682 Bustamante, M. M. C., Nobre, C. A., Smeraldi, R., Aguiar, A. P. D., Barioni, L. G., Ferreira, L.
683 G., Longo, K., May, P., Pinto, A. S., & Ometto, J. P. H. B. (2012). Estimating greenhouse
684 gas emissions from cattle raising in Brazil. *Climatic Change*, 115(3–4), 559–577.
685 <https://doi.org/10.1007/s10584-012-0443-3>

686 Butterbach-Bahl, K., Rothe, A., & Papen, H. (2002). Effect of tree distance on N₂O and CH₄-
687 fluxes from soils in temperate forest ecosystems. *Plant and Soil*, 240(1), 91–103.
688 <https://doi.org/10.1023/A:1015828701885>

689 Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should
690 replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*,
691 11(12), 2639–2643. <https://doi.org/10.1038/ismej.2017.119>

692 Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-Lyons, D., Lozupone, C. A., Turnbaugh,
693 P. J., Fierer, N., & Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of
694 millions of sequences per sample. *Proceedings of the National Academy of Sciences of*
695 *the United States of America*, 108 Suppl 1(Supplement 1), 4516–4522.
696 <https://doi.org/10.1073/pnas.1000080107>

697 Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., Brown, C. T., Porras-
698 Alfaro, A., Kuske, C. R., & Tiedje, J. M. (2014). Ribosomal Database Project: data and
699 tools for high throughput rRNA analysis. *Nucleic Acids Research*, 42(Database issue),
700 D633–D642. <https://doi.org/10.1093/nar/gkt1244>

701 Costello, A. M., & Lidstrom, M. E. (1999). Molecular Characterization of Functional and
702 Phylogenetic Genes from Natural Populations of Methanotrophs in Lake Sediments.
703 *Applied and Environmental Microbiology*, 65(11), 5066–5074.
704 <http://aem.asm.org/content/65/11/5066>

705 de Carvalho, T. S., Jesus, E. da C., Barlow, J., Gardner, T. A., Soares, I. C., Tiedje, J. M., &
706 Moreira, F. M. de S. (2016). Land use intensification in the humid tropics increased both
707 alpha and beta diversity of soil bacteria. *Ecology*, 97(10), 2760–2771.
708 <https://doi.org/10.1002/ecy.1513>

709 de Moraes, J. F. L., Volkoff, B., Cerri, C. C., & Bernoux, M. (1996). Soil properties under
710 Amazon forest and changes due to pasture installation in Rondônia, Brazil. *Geoderma*,
711 70(1), 63–81. [https://doi.org/10.1016/0016-7061\(95\)00072-0](https://doi.org/10.1016/0016-7061(95)00072-0)

712 Demattê, J. L. I., & Demattê, J. A. M. (1993). Comparações entre as propriedades químicas
713 de solos das regiões da floresta amazônica e do cerrado do Brasil central. *Sci. agric.*,
714 50(2), 272-286. <https://doi.org/10.1590/S0103-90161993000200016>.

715 Dias, L. C., Pimenta, F. M., Santos, A. B., Costa, M. H., Ladle R. J. (2016). Patterns of land
716 use, extensification, and intensification of Brazilian agriculture. *Glob. Change*
717 *Biol.*, 22 (8), pp. 2887-2903.

718 Dias-Filho, M. B. (2017). Degradação de pastagens: o que é e como evitar. Embrapa,
719 Brasília, DF, Brazil. ISBN 978-85-7035-688-8.

720 Domeignoz-Horta, L. A., Philippot L., Peyrard C., *et al* (2018). Peaks of in situ N₂O emissions
721 are influenced by N₂O producing and reducing microbial communities across arable
722 soils. *Glob. Change Biol.*, 24: 360-370. <https://doi.org/10.1111/gcb.13853>

723 Evans, P. N., Boyd, J. A., Leu, A. O. *et al.* (2019). An evolving view of methane metabolism in
724 the Archaea. *Nat Rev Microbiol* 17, 219–232. <https://doi.org/10.1038/s41579-018-0136-7>

725 Fearnside, P. M., & Imbrozio Barbosa, R. (1998). Soil carbon changes from conversion of
726 forest to pasture in Brazilian Amazonia. *Forest Ecology and Management*, 108(1–2),
727 147–166. [https://doi.org/10.1016/S0378-1127\(98\)00222-9](https://doi.org/10.1016/S0378-1127(98)00222-9)

728 Fedarko, M. W., Martino, C., Morton, J. T., González, A., Rahman, G., Marotz, C. A., Minich,
729 J. J., Allen, E. E., & Knight, R. (2019). Visualizing 'omic feature rankings and log-ratios
730 using Qurro. *BioRxiv*, 2019.12.17.880047. <https://doi.org/10.1101/2019.12.17.880047>

731 Fernandes, S. A. P., Bernoux, M., Cerri, C. C., Feigl, B. J., & Piccolo, M. C. (2002). Seasonal
732 variation of soil chemical properties and CO₂ and CH₄ fluxes in unfertilized and P-
733 fertilized pastures in an Ultisol of the Brazilian Amazon. *Geoderma*, 107(3–4), 227–241.
734 [https://doi.org/10.1016/S0016-7061\(01\)00150-1](https://doi.org/10.1016/S0016-7061(01)00150-1)

735 Fierer, N., & Jackson, R. B. (2006). The diversity and biogeography of soil bacterial
736 communities. *Proceedings of the National Academy of Sciences of the United States of*
737 *America*, 103(3), 626–631. <https://doi.org/10.1073/pnas.0507535103>

738 Figueiredo, E. B., Jayasundara, S., Bordonal, R. O., Berchielli, T. T., Reis, R. A., Wagner-
739 Riddle, C., La Scala, N. (2017). Greenhouse gas balance and carbon footprint of beef
740 cattle in three contrasting pasture-management systems in Brazil. *Journal of Cleaner*
741 *Production*, 142 (1), 420-431.

742 Forster, P., V. Ramaswamy, P. Artaxo, T. Berntsen, R. Betts, D.W. Fahey, J. Haywood, J.
743 Lean, D.C. Lowe, G. Myhre, J. Nganga, R. P., & G. Raga, M. S. and R. V. D. (2007).
744 Changes in Atmospheric Constituents and in Radiative Forcing. In M. T. and H. L. M.
745 Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt (Ed.), *Climate*

746 *Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth*
747 *Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge
748 University Press. [http://www.cgd.ucar.edu/events/20130729/files/Forster-Ramaswamy-](http://www.cgd.ucar.edu/events/20130729/files/Forster-Ramaswamy-etal-2007.pdf)
749 [etal-2007.pdf](http://www.cgd.ucar.edu/events/20130729/files/Forster-Ramaswamy-etal-2007.pdf)

750 Frenzel, P., Rothfuss, F., & Conrad, R. (1992). Oxygen profiles and methane turnover in a
751 flooded rice microcosm. *Biology and Fertility of Soils*, 14(2), 84–89.
752 <https://doi.org/10.1007/BF00336255>

753 García-Marco, S., Abalos, D., Espejo, R., Vallejo, A., & Mariscal-Sancho, I. (2016). No tillage
754 and liming reduce greenhouse gas emissions from poorly drained agricultural soils in
755 Mediterranean regions. *Science of The Total Environment*, 566, 512–520. [https://doi.org/](https://doi.org/10.1016/j.scitotenv.2016.05.117)
756 [10.1016/j.scitotenv.2016.05.117](https://doi.org/10.1016/j.scitotenv.2016.05.117)

757 Hanson, R. S., & Hanson, T. E. (1996). Methanotrophic bacteria. *Microbiological Reviews*,
758 60(2), 439–471. <http://www.ncbi.nlm.nih.gov/pmc/articles/PMC239451/>

759 Herlemann, D. P., Labrenz, M., Jürgens, K., Bertilsson, S., Waniek, J. J., & Andersson, A. F.
760 (2011). Transitions in bacterial communities along the 2000 km salinity gradient of the
761 Baltic Sea. *The ISME Journal*, 5(10), 1571–1579. <https://doi.org/10.1038/ismej.2011.41>

762 Herpin, U., Cerri, C. C., Conceição Santana Carvalho, M., Markert, B., Enzweiler, J., Friese,
763 K., & Breulmann, G. (2002). Biogeochemical dynamics following land use change from
764 forest to pasture in a humid tropical area (Rondônia, Brazil): a multi-element approach by
765 means of XRF-spectroscopy. *Science of The Total Environment*, 286(1–3), 97–109.
766 [https://doi.org/10.1016/S0048-9697\(01\)00967-6](https://doi.org/10.1016/S0048-9697(01)00967-6)

767 Herrero, M., Henderson, B., Havlík, P. *et al.* (2016). Greenhouse gas mitigation potentials in
768 the livestock sector. *Nature Clim Change* 6, 452–461.
769 <https://doi.org/10.1038/nclimate2925>

770 Holmes, A. J., Roslev, P., McDonald, I. R., Iversen, N., Henriksen, K., & Murrell, J. C. (1999).
771 Characterization of Methanotrophic Bacterial Populations in Soils Showing Atmospheric
772 Methane Uptake. *Applied and Environmental Microbiology*, 65(8), 3312–3318.
773 <http://aem.asm.org/content/65/8/3312>

774 Hütsch, B. W., Webster, C. P., & Powlson, D. S. (1994). Methane oxidation in soil as affected
775 by land use, soil pH and N fertilization. *Soil Biology and Biochemistry*, 26(12), 1613–
776 1622. [https://doi.org/10.1016/0038-0717\(94\)90313-1](https://doi.org/10.1016/0038-0717(94)90313-1)

777 IPCC, I. P. O. C. C. (2007). *Contribution of Working Groups I, II and III to the Fourth*
778 *Assessment Report of the Intergovernmental Panel on Climate Change* (L. A. M. B. Metz,
779 O.R. Davidson, P.R. Bosch, R. Dave (ed.)). Cambridge University Press.

780 Jesus, E. da C., Marsh, T. L., Tiedje, J. M., & Moreira, F. M. de S. (2009). Changes in land
781 use alter the structure of bacterial communities in Western Amazon soils. *The ISME*
782 *Journal*, 3(9), 1004–1011. <https://doi.org/10.1038/ismej.2009.47>

783 Kaimowitz, D., Mertens, B., Wunder, S., & Pacheco, P. (n.d.). *Cattle ranching and*
784 *deforestation in Brazil's Amazon CENTER FOR INTERNATIONAL FORESTRY*
785 *RESEARCH*. Retrieved February 15, 2020, from
786 <http://earthtrends.wri.org/text/AGR/variables/193.htm>

787 Kallistova, A. Y., Merkel, A. Y., Tarnovetskii, I. Y., & Pimenov, N. V. (2017). Methane
788 formation and oxidation by prokaryotes. *Microbiology*, 86(6), 671–691.
789 <https://doi.org/10.1134/S0026261717060091>

790 Keppler, F., Hamilton, J. T. G., Braß, M., & Röckmann, T. (2006). Methane emissions from
791 terrestrial plants under aerobic conditions. *Nature*, 439(7073), 187–191.
792 <https://doi.org/10.1038/nature04420>

793 Kimura, M. (1980). A simple method for estimating evolutionary rates of base substitutions
794 through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*,
795 16(2), 111–120. <https://doi.org/10.1007/BF01731581>

796 Knief, C. (2015). Diversity and Habitat Preferences of Cultivated and Uncultivated Aerobic
797 Methanotrophic Bacteria Evaluated Based on pmoA as Molecular Marker. *Terrestrial*
798 *Microbiology*, 1346. <https://doi.org/10.3389/fmicb.2015.01346>

799 Knief, C., Lipski, A., & Dunfield, P. F. (2003). Diversity and Activity of Methanotrophic Bacteria
800 in Different Upland Soils. *Applied and Environmental Microbiology*, 69(11), 6703–6714.
801 <https://doi.org/10.1128/AEM.69.11.6703-6714.2003>

802 Kolb, S. (2009). The quest for atmospheric methane oxidizers in forest soils. *Environmental*
803 *Microbiology Reports*, 1(5), 336–346. <https://doi.org/10.1111/j.1758-2229.2009.00047.x>

804 Kroeger, M.E., Meredith, L.K., Meyer, K.M. *et al.* Rainforest-to-pasture conversion stimulates
805 soil methanogenesis across the Brazilian Amazon. *ISME J* (2020).
806 <https://doi.org/10.1038/s41396-020-00804-x>

807 Kunhikrishnan, A., Thangarajan, R., Bolan, N.S., Xu, Y., Mandal, S., Gleeson, D.B., Seshadri,
808 B., Zaman, M., Barton, L., Tang, C., Luo, J., Dalal, R., Ding, W., Kirkham, M.B., Naidu,
809 R., 2016. Functional Relationships of Soil Acidification, Liming, and Greenhouse Gas
810 Flux, in: Sparks, D. (Ed.), ADVANCES IN AGRONOMY, VOL 139, Advances in
811 Agronomy. ELSEVIER ACADEMIC PRESS INC, 525 B STREET, SUITE 1900, SAN
812 DIEGO, CA 92101-4495 USA, pp. 1–71. <https://doi.org/10.1016/bs.agron.2016.05.001>

813 Lal, R. (2001). Soil degradation by erosion. *Land Degradation and Development*, 12, 519-539.

814 Lammel, D. R., Barth, G., Ovaskainen, O., Cruz, L. M., Zanatta, J. A., Ryo, M., de Souza, E.
815 M., & Pedrosa, F. O. (2018). Direct and indirect effects of a pH gradient bring insights
816 into the mechanisms driving prokaryotic community structures. *Microbiome*, 6(1), 106.
817 <https://doi.org/10.1186/s40168-018-0482-8>

818 Lau, J.A. & Lennon, J.T. (2011). Evolutionary ecology of plant–microbe interactions: soil
819 microbial structure alters selection on plant traits. *New Phytologist* 192, 215–224.
820 <https://doi.org/10.1111/j.1469-8137.2011.03790.x>

821 Laurance, W. F., Laurance, S. G., & Delamonica, P. (1998). Tropical forest fragmentation and
822 greenhouse gas emissions. *Forest Ecology and Management*, 110(1–3), 173–180.
823 [https://doi.org/10.1016/S0378-1127\(98\)00291-6](https://doi.org/10.1016/S0378-1127(98)00291-6)

824 Le Mer, J., & Roger, P. (2001). Production, oxidation, emission and consumption of methane
825 by soils: A review. *European Journal of Soil Biology*, 37(1), 25–50.
826 [https://doi.org/10.1016/S1164-5563\(01\)01067-6](https://doi.org/10.1016/S1164-5563(01)01067-6)

827 Liu, C., Holst, J., Brüggemann, N., Butterbach-Bahl, K., Yao, Z., Yue, J., Han, S., Han, X.,
828 Krümmelbein, J., Horn, R., & Zheng, X. (2007). Winter-grazing reduces methane uptake
829 by soils of a typical semi-arid steppe in Inner Mongolia, China. *Atmospheric Environment*,
830 41(28), 5948–5958. <https://doi.org/10.1016/j.atmosenv.2007.03.017>

831 Margulis, S. (2003). *Causes of Deforestation of the Brazilian Amazon*. The World Bank.
832 <https://doi.org/10.1596/0-8213-5691-7>

833 Martino, C., Morton, J. T., Marotz, C. A., Thompson, L. R., Tripathi, A., Knight, R., & Zengler,
834 K. (2019). A Novel Sparse Compositional Technique Reveals Microbial Perturbations.
835 *MSystems*, 4(1). <https://doi.org/10.1128/msystems.00016-19>

836 Mendes, L. W., Tsai, S. M., Navarrete, A. A., Hollander, M. de, Veen, J. A. van, & Kuramae,
837 E. E. (2015). Soil-Borne Microbiome: Linking Diversity to Function. *Microbial Ecology*,
838 70(1), 255–265. <https://doi.org/10.1007/s00248-014-0559-2>

839 Meyer, K. M., Klein, A. M., Rodrigues, J. L. M., Nüsslein, K., Tringe, S. G., Mirza, B. S.,
840 Tiedje, J. M., & Bohannan, B. J. M. (2017). Conversion of Amazon rainforest to
841 agriculture alters community traits of methane-cycling organisms. *Molecular Ecology*,
842 26(6), 1547–1556. <https://doi.org/10.1111/mec.14011>

843 Meyer, K.M., Morris, A.H., Webster, K., Klein, A.M., Kroeger, M.E., Meredith, L.K. Brændholt,
844 A., Nakamura, F., Venturini, A., de Souza, L.F., Shek, k. L., Danielson, R., van Haren, J.,
845 de Camargo, P.B., Tsai, S.M., Dini-Andreote, F., Mauro, J., Barlow, J., Berenguer, E.,
846 Nüsslein, K., Saleska, S., Rodrigues, J.L.M., Bohannan, B.J.M. (2020). Belowground
847 changes to community structure alter methane-cycling dynamics in Amazonia.
848 *Environment International*, 145, 106131.

849 Miguez, C. B., Bourque, D., Sealy, J. A., Greer, C. W., & Groleau, D. (n.d.). Detection and
850 Isolation of Methanotrophic Bacteria Possessing Soluble Methane Monooxygenase
851 (sMMO) Genes Using the Polymerase Chain Reaction (PCR). *Microbial Ecology*, 33(1),
852 21–31. <https://doi.org/10.1007/s002489900004>

853 Morton, D. C., DeFries, R. S., Shimabukuro, Y. E., Anderson, L. O., Arai, E., Del Bon Espirito-
854 Santo, F., Freitas, R., & Morissette, J. (2006). Cropland expansion changes deforestation
855 dynamics in the southern Brazilian Amazon. *Proceedings of the National Academy of*
856 *Sciences of the United States of America*, 103(39), 14637–14641.
857 <https://doi.org/10.1073/pnas.0606377103>

858 Mosier, A. R., Delgado, J. A., & Keller, M. (1998). Methane and nitrous oxide fluxes in an acid
859 Oxisol in western Puerto Rico: effects of tillage, liming and fertilization. *Soil Biology and*
860 *Biochemistry*, 30(14), 2087–2098. [https://doi.org/10.1016/S0038-0717\(98\)00085-6](https://doi.org/10.1016/S0038-0717(98)00085-6)

861 Navarrete, Acacio A., Tsai, S. M., Mendes, L. W., Faust, K., de Hollander, M., Cassman, N.
862 A., Raes, J., van Veen, J. A., & Kuramae, E. E. (2015). Soil microbiome responses to the
863 short-term effects of Amazonian deforestation. *Molecular Ecology*, 24(10), 2433–2448.
864 <https://doi.org/10.1111/mec.13172>

865 Nazaries, L., Murrell, J. C., Millard, P., Baggs, L., & Singh, B. K. (2013). Methane, microbes
866 and models: Fundamental understanding of the soil methane cycle for future predictions.

867 In *Environmental Microbiology* (Vol. 15, Issue 9). <https://doi.org/10.1111/1462->
868 2920.12149

869 Oliveira, P. P. A., Boaretto, A. E., Trivelin, P. C. O., de Oliveira, W. S., Corsi, M. (2003).
870 Liming and fertilization to restore degraded *Brachiaria decumbens* pastures grown on an
871 entisol. *Sci. Agric.*, 60 (1), 125-131.

872 Oksanen, J., Blanchet, F. G., Kindt, R., & Wagner, L. H. (2015). Vegan: Community ecology
873 package. R package version 2.2-1. Available online at:
874 <http://CRAN.R-project.org/package=vegan>

875 Parada, A. E., Needham, D. M., & Fuhrman, J. A. (2016). Every base matters: assessing
876 small subunit rRNA primers for marine microbiomes with mock communities, time series
877 and global field samples. *Environmental Microbiology*, 18(5), 1403–1414.
878 <https://doi.org/10.1111/1462-2920.13023>

879 Peres-Neto, P. R., Legendre, P., Dray, S., & Borcard, D. (2006). Variation partitioning of
880 species data matrices: Estimation and comparison of fractions. *Ecology*, 87, 2614–2625.

881 Philippot, L., Hallin, S., Börjesson, G., & Baggs, E. M. (2009). Biochemical cycling in the
882 rhizosphere having an impact on global change. In *Plant and Soil* (Vol. 321, Issues 1–2,
883 pp. 61–81). <https://doi.org/10.1007/s11104-008-9796-9>

884 Philippot, Laurent, Raaijmakers, J. M., Lemanceau, P., & van der Putten, W. H. (2013). Going
885 back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology*,
886 11(11), 789–799. <https://doi.org/10.1038/nrmicro3109>

887 Praeg, N., Wagner, A. O., & Illmer, P. (2017). Plant species, temperature, and bedrock affect
888 net methane flux out of grassland and forest soils. *Plant and Soil*, 410(1–2), 193–206.
889 <https://doi.org/10.1007/s11104-016-2993-z>

890 Pratscher, J., Vollmers, J., Wiegand, S., Dumont, M. G., & Kaster, A.-K. (2018). Unravelling
891 the Identity, Metabolic Potential and Global Biogeography of the Atmospheric Methane-
892 Oxidizing Upland Soil Cluster α . *Environmental Microbiology*, n/a–n/a.
893 <https://doi.org/10.1111/1462-2920.14036>

894 Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., &
895 Glöckner, F. O. (2012). The SILVA ribosomal RNA gene database project: improved data
896 processing and web-based tools. *Nucleic Acids Research*, 41(D1), D590–D596.
897 <https://doi.org/10.1093/nar/gks1219>

898 Ramakers, C., Ruijter, J. M., Lekanne Deprez, R. H., & Moorman, A. F. M. (2003).
899 Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data.
900 *Neuroscience Letters*, 339(1), 62–66. [https://doi.org/10.1016/S0304-3940\(02\)01423-4](https://doi.org/10.1016/S0304-3940(02)01423-4)

901 Reiners, W. A., Bouwman, A. F., Parsons, W. F. J., & Keller, M. (1994). Tropical Rain Forest
902 Conversion to Pasture: Changes in Vegetation and Soil Properties. *Ecological*
903 *Applications*, 4(2), 363–377. <https://doi.org/10.2307/1941940>

904 Rodrigues, J. L. M., Pellizari, V. H., Mueller, R., Baek, K., Jesus, E. da C., Paula, F. S., Mirza,
905 B., Hamaoui, G. S., Tsai, S. M., Feigl, B., Tiedje, J. M., Bohannan, B. J. M., & Nüsslein,
906 K. (2013). Conversion of the Amazon rainforest to agriculture results in biotic
907 homogenization of soil bacterial communities. *Proceedings of the National Academy of*
908 *Sciences*, 110(3), 988–993. <https://doi.org/10.1073/pnas.1220608110>

909 Segnini, Aline, Xavier, Alfredo Augusto Pereira, Otaviani-Junior, Pedro Luis, Oliveira, Patrícia
910 Perondi Anchão, Pedroso, André de Faria, Praes, Maria Fernanda Ferreira Menegucci,
911 Rodrigues, Paulo Henrique Mazza, & Milori, Débora Marcondes Bastos Pereira. (2019).
912 Soil carbon stock and humification in pastures under different levels of intensification in
913 Brazil. *Scientia Agricola*, 76(1), 33-40. <https://doi.org/10.1590/1678-992x-2017-0131>

914 Semrau, J.D., Chistoserdov, A., Lebron, J., Costello, A., Davagnino, J., Kenna, E., Holmes,
915 A.J., Finch, R., Murrell, J.C., Lidstrom, M.E., 1995. Particulate methane monooxygenase
916 genes in methanotrophs. *J. Bacteriol.* 177, 3071–3079.

917 Serrano-Silva, N., Sarria-Guzmán, Y., Dendooven, L., & Luna-Guido, M. (2014).
918 Methanogenesis and Methanotrophy in Soil: A Review. *Pedosphere*, 24(3), 291–307.
919 [https://doi.org/10.1016/S1002-0160\(14\)60016-3](https://doi.org/10.1016/S1002-0160(14)60016-3)

920 Steinberg, L. M., & Regan, J. M. (2008). Phylogenetic Comparison of the Methanogenic
921 Communities from an Acidic, Oligotrophic Fen and an Anaerobic Digester Treating
922 Municipal Wastewater Sludge. *Applied and Environmental Microbiology*, 74(21), 6663–
923 6671. <https://doi.org/10.1128/AEM.00553-08>

924 Steudler, P. A., Melillo, J. M., Feigl, B. J., Neill, C., Piccolo, M. C., & Cerri, C. C. (1996).
925 Consequence of forest-to-pasture conversion on CH₄ fluxes in the Brazilian Amazon
926 Basin. *Journal of Geophysical Research: Atmospheres*, 101(D13), 18547–18554. <https://doi.org/10.1029/96JD01551>

927

928 Tamas, I., Smirnova, A. V., He, Z., & Dunfield, P. F. (2014). The (d)evolution of
 929 methanotrophy in the Beijerinckiaceae—a comparative genomics analysis. *ISME Journal*,
 930 8(2), 369–382. <https://doi.org/10.1038/ismej.2013.145>

931 Tate, K. R. (2015). Soil methane oxidation and land-use change – from process to mitigation.
 932 *Soil Biology and Biochemistry*, 80, 260–272. <https://doi.org/10.1016/j.soilbio.2014.10.010>

933 Tian, H., Chen, G., Lu, C., Xu, X., Ren, W., Zhang, B., Banger, K., Tao, B., Pan, S., Liu, M.,
 934 Zhang, C., Bruhwiler, L., & Wofsy, S. (2015). Global methane and nitrous oxide
 935 emissions from terrestrial ecosystems due to multiple environmental changes.
 936 *Ecosystem Health and Sustainability*, 1(1), 1–20. <https://doi.org/10.1890/EHS14-0015.1>

937 Ussiri, D.A.N., Lal, R., Jarecki, M.K. (2009). Nitrous oxide and methane emissions from long-
 938 term tillage under a continuous corn cropping system in Ohio. *Soil and Tillage Research*,
 939 104(2), 247-255.

940 van Raij, B., de; Andrade, J.C., Cantarella, H. & Quaggio, J.A. (2001). Análise química para
 941 avaliação da fertilidade de solos tropicais. Campinas, Brazil.

942 Verchot, L. V., Davidson, E. A., Cattânio, J. H., & Ackerman, I. L. (2000). Land-Use Change
 943 and Biogeochemical Controls of Methane Fluxes in Soils of Eastern Amazonia.
 944 *Ecosystems*, 3(1), 41–56. <https://doi.org/10.1007/s100210000009>

945 Wang Y, Yao Z, Zhan Y, Zheng X, Zhou M, Yan G, Wang L, Werner C, Butterbach-Bahl K.
 946 Potential benefits of liming to acid soils on climate change mitigation and food security.
 947 *Global Change Biology*. 2021 Mar 20. <https://doi.org/10.1111/gcb.15607>

948 Whittenbury, R., Davies, S. L., & Davey, J. F. (1970). Exospores and Cysts Formed by
 949 Methane-utilizing Bacteria. *Microbiology*, 61(2), 219–226.
 950 <https://doi.org/10.1099/00221287-61-2-219>

951