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Conversion of Amazon rainforest to agriculture alters community traits of methane-cycling organisms

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ABSTRACT

Land use change is one of the greatest environmental threats worldwide, especially to tropical forests. The Amazon rainforest has been subject to particularly high rates of land use change, primarily to cattle pasture. A commonly observed response to cattle pasture establishment in the Amazon is the conversion of soil from a methane sink in rainforest, to a methane source in pasture. However, it is not known how the microorganisms that mediate methane flux are altered by land use change. Here we use the deepest metagenomic sequencing of Amazonian soil to date to investigate differences in methane-cycling microorganisms and their traits across rainforest and cattle pasture soils. We found that methane-cycling microorganisms responded to land use change, with the strongest responses exhibited by methane-consuming, rather than methane-producing, microorganisms. These responses included a reduction in the relative abundance of methanotrophs and a significant decrease in abundance of genes encoding particulate methane monooxygenase (pMMO). We also observed compositional changes to methanotrophs and methanogens as well as changes to methanotroph life history strategies. Our observations suggest that methane-cycling organisms are vulnerable to land use change, and this vulnerability may underlie the response of methane flux to land use change in Amazon soils.

INTRODUCTION

Land use change poses one of the largest threats to global biodiversity[1]. In tropical regions, this process is occurring disproportionately faster than in any other region

worldwide[2,3]. In the Amazon Basin, the primary motivation for land use change is conversion to cattle pasture. This process has been shown to alter soil chemistry[4–6], as well as soil microbial biodiversity and functional traits[5,7,8] , leading to alteration of a number of ecosystem processes governed by soil microbes such as methane emission[9,10].

Methane is a potent greenhouse gas with a global warming potential that is 34 times higher than CO_2 (over a 100-year time frame)[11]. It is well established that forest soils throughout the Amazon Basin generally act as methane sinks[9,10,12,13]. But when forests in the Amazon Basin are converted to cattle pasture, the underlying soils can shift from methane sink to source[9,10,12,13]. It is not known what factors are responsible for this shift. Most of the proposed explanations have focused on physico-chemical alterations to the soil (e.g. increased water-filled pore space and decreased O_2 diffusion) driving increased methanogenesis[10,12], yet few have investigated how the conversion process alters the communities and traits of microorganisms responsible for these processes.

Two counteracting microbial processes control biogenic methane emission: methanogenesis and methanotrophy. These processes are both governed by a suite of phylogenetically conserved community traits[14]. Methane flux rates have been associated with the community composition[15–18], abundance, and activity[19,20] of both methanogens and methanotrophs. Thus, there is precedent to suggest that shifts in methane-cycling community traits could alter rates of methane flux. Moreover, each of these functional groups can be further divided by differences in specific traits. Within the methanotrophs, Type I methanotrophs, Type II methanotrophs, and methanotrophic Verrucomicrobia differ in their physiology, substrate affinity, and life history strategies[21,22]. Within the methanogens, acetoclastic, hydrogenotrophic, and methylotrophic taxa display different life history strategies, utilize different substrates for methanogenesis, and can generate methane at different rates[23,24].

Applying trait-based approaches to microbial ecology can provide an alternative perspective on community responses to environmental change[25]. Such approaches can reveal, for example, shifts in functional potential (*i.e.* gene content), taxonomy (e.g. diversity or composition), or life history strategies[26,27]. Life history strategies generally refer to an organism's investments in survival, growth, and reproduction. Documenting changes in life history strategies has played an important role in understanding how plant and animal communities respond to environmental change, but this approach has rarely been applied to microbial communities[28] mainly due to the difficulty of cultivating the majority of microbial taxa. Ho et al. (2013)[22] classified methanotrophic microorganisms into the Competitor-Stress tolerator-Ruderal life history framework[29] using physiological measurements of cultured representatives and habitat range data. This framework divides taxa among three primary strategies: "Competitors" (exhibiting fast growth under high nutrient or substrate conditions), "Stress Tolerators" (tolerating low or variable substrate availability), and "Ruderals" (performing optimally in frequently disturbed sites). Under this system, Type II methanotrophs are primarily classified as Stress Tolerators – performing better under conditions of low or variable methane or O_2 availability, while Type I methanotrophs are more variable, spanning from Competitor to Ruderal- implying that they perform better under conditions of high substrate availability or in frequently disturbed sites. Using a trait-based framework such as this provides new insights into how microbial communities respond to environmental change, and provides a more thorough understanding of the contribution of community attributes to ecosystem functioning.

Here we apply a trait and life history-based framework to ask how methane-cycling communities differ between primary rainforest and cattle pasture derived from primary rainforest in the Western Amazon. We use environmental metagenomics to provide a more comprehensive assessment of microbial community traits than can be obtained by culture-dependent methods or culture-independent methods which rely on sequencing of individual target genes. First we investigate changes in the abundances of methane-cycling taxa, their functional traits, and their life history strategies. Secondly, we compare the abundance of genes involved in methane-cycling pathways. We show that traits for both processes vary across the two land-types but that methanotroph traits exhibit a more pronounced change. Finally, we discuss how changes to these community traits are consistent with the shift from methane-sink to source previously reported from this site.

MATERIALS AND METHODS

Site Description and Sampling

Our study was performed at the Amazon Rainforest Microbial Observatory (ARMO) site (10°10'5'' S and 62°49'27'' W)[4]. This site was selected as representative of the current agricultural expansion in the Western Amazon. It is located in the Brazilian state of Rondônia, which has experienced the highest percentage of forest loss (28%) of any state in the Brazilian Amazon. Agricultural conversion in this region typically follows the following stages: 1) selective logging of valuable timber, 2) slash-and-burn deforestation of the remaining vegetation, and 3) aerial seeding of members of the non-native fast-growing grass genera *Urochloa* (formerly *Brachiara*) or *Panicum* in order to establish pasture for cattle ranching. Pastures may be burned periodically in order to control the invasion of weeds. Herbicides, tillage, or chemical fertilizers are not commonly used. The vegetation and soil characteristics at this site have been described in detail elsewhere[30,31].

Ten soil cores were collected from ARMO in April 2010 (5 soil cores from primary rainforest and 5 from a 42 year-old converted pasture). Soil was sampled to a depth of 10 cm (after removal of the litter layer) using standard coring methods and homogenized. Samples were frozen on the spot, transported on dry ice, and stored at -80° C until extraction.

Soil DNA Extraction and Sequencing

DNA was extracted from soils following the same protocol as described in Rodrigues et al. (2013)[4]. Illumina TruSeq libraries with ~270 bp insert sizes were constructed from 10 samples according to standard protocol, and sequenced with 150 bp paired-end reads on the Illumina HiSeq. In total, 21 lanes were sequenced to produce 6.4 billion paired-end reads, resulting in an average of 636 million (±12%) reads per sample. This depth gives us a unique opportunity to investigate a variety of relatively rare genetic markers for methane cycling.

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Bioinformatics and Statistics

Functional and taxonomic annotations were obtained using the MG-RAST pipeline[32]. Raw sequences were uploaded to MG-RAST, and paired-end reads were joined using fastq-join as part of the MG-RAST pipeline. Single end reads that could not be joined were retained. After merging paired-end reads, a total of 6.3 billion sequences with an average length of 171 bp were processed through the MG-RAST pipeline. All other pipeline options

were left as default (*i.e.* trimming of low quality bases, removal of artificial replicate sequences, and filtering of sequences with greater than 5 ambiguous bases). Hierarchical functional annotations were generated using the SEED subsystems[33] and organismal annotations were obtained via the MG-RAST M5RNA database[34] which assigns taxonomy strictly from ribosome-encoding genes including those from the SILVA, RDP, and Greengenes databases[35]. We used the "Representative Hit" classification method for organismal annotation, which selects a single, unambiguous annotation for each feature and assigns taxonomy. Default parameters (e-value cutoff = 1e-5, Min. % identity = 60%, Min. alignment length cutoff = 15) were used for both the functional and taxonomic annotations. 16.4% of sequences were removed for low quality. 0.5% of sequences were rRNA genes, 21.7% were predicted proteins with known functions, 47.9% predicted proteins with unknown functions, and 13.5% did not contain rRNA genes or predicted proteins. We obtained usable annotations for approximately 22.2% of the total sequences (0.5% rRNA genes + 21.7% predicted proteins with known functions).

The functional annotations had an average of 1.2 million ± 136,000 observations per sample. The organismal annotations had an average of 304,000 ± 76,000 observations per sample. Annotation tables were subsampled to achieve equal sampling depth across samples. The functional annotation table was rarefied to 1.05 million observations per sample and the organismal table was rarefied to 195,000 observations per sample. All analyses were performed using the R statistical environment[36] including the vegan package[37].

We constructed functional group community matrices by selecting only species previously reported in the literature as methanotrophs or methanogens (Supplementary Tables 1 & 2, respectively). Community composition differences were statistically tested using a permutational multivariate ANOVA (PERMANOVA) on Bray Curtis[38] and Canberra[39] community distances. Shannon diversity, species richness, Simpson diversity, and Pielou's evenness were used to assess varying aspects of functional group diversity and were compared across sites using a two-sample two-tailed *t-test*. The abundance of genes encoding methane-cycling functions were compared across samples using a two-sample two-tailed *t-test*. Comparison of ratios across land types was performed by first log transforming the ratios then testing using a two-sample one-tailed *t-test*.

Methanotroph genera were characterized under the Competitor-Stress tolerator-Ruderal functional classification[29] proposed for methanotrophs by Ho et al. (2013)[22] (Supplementary Table 3). The relative abundances of each of these functional groups were compared using a two-sample two-tailed *t*-test. Ratios of these groups were first log-transformed then compared using a two-sample two-tailed *t*-test.

RESULTS & DISCUSSION

Shifts in the methanotroph-to-methanogen ratio

Methane flux is the net balance between methane production and methane consumption. Thus changes to the balance of the organisms that mediate these processes, or genes encoding the cellular machinery by which these processes take place, could alter methane flux. The forest site at which our study was conducted has been shown to exhibit net negative methane flux (methane consumption), even in the wet season[10]. By contrast, the cattle pasture where we sampled was shown to exhibit positive methane flux (methane

emission), even in the dry season[10]. One of our primary findings is that there were fewer methanotroph sequences per methanogen sequence in the pasture relative to the forest (Fig. 1a, Forest average ratio 84.7±37.3, Pasture 38.8±17.1, $P < 0.05$). This was driven by a significant decrease in methanotroph abundance in the pasture and no significant change in the methanogen abundance across land types. Moreover, we estimated the ratio of potential methanotrophy to methanogenesis by comparing the abundance of genes encoding methane monooxygenases (MMO) and methyl coenzyme M reductase (MCR). This ratio shows a similar trend (Fig. 1b), driven by a decrease in MMO gene abundance and an increase in MCR gene abundance. A shift in these ratios could impact the amount of methane consumed relative to the amount of methane produced, leading to changes in the net flux of methane from the soil.

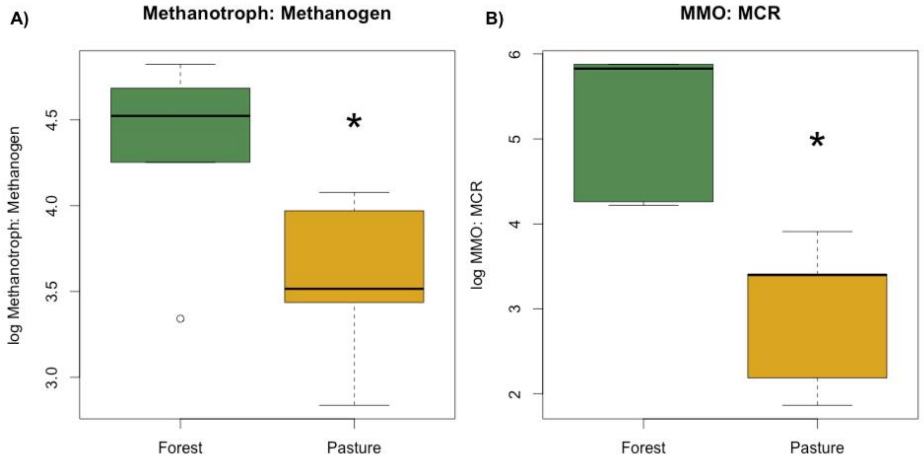

Figure 1: Methanotroph-to-methanogen ratios differ in forest and pasture. A. Ratio calculated from rRNA organismal annotations. B. The ratio of the abundance of methane monooxygenase (MMO; a methanotrophy marker) to methyl-coenzyme M reductase (MCR; a methanogenesis marker) gene annotations. Significant differences between forest and pasture are denoted as: * $P < 0.05$.

Shifts in taxonomic groups

Our annotations recovered sequences from Type I (Gammaproteobacteria), Type II (Alphaproteobacteria), and Verrucomicrobia methanotrophs (Supplementary Table 1), as well as methanogen sequences from the orders *Methanobacteriales, Methanococcales, Methanocellales, Methanomicrobiales, Methanosarcinales*, and *Methanopyrales* (Supplementary Table 2).

Methanotrophic taxa. We observed numerous changes to methanotroph community traits that suggest that methanotrophy could be altered following conversion of forest to pasture. Methanotroph communities were compositionally different across the two land types (Bray-Curtis R^2=0.41, P<0.05; Canberra R^2=0.17, P<0.05). The forest community exhibited a significantly higher average pairwise dissimilarity than the pasture community

(Bray-Curtis $P<0.05$; Canberra $P<0.05$), indicating a higher variability in community membership in the forest. The methanotroph community did not differ in richness, Shannon diversity, Simpson diversity, or evenness across land types. However, when the Proteobacteria methanotrophs (only Type I and Type IIs) were considered separately, Shannon diversity, Simpson diversity, and evenness were significantly higher in the pasture ($P < 0.05$).

Of the three main types of methanotrophs at our sites (Type I, II, and Verrucomicrobia), the Type II methanotrophs vary more between the land types (Fig. 2). Neither Type I nor Verrucomicrobia methanotroph communities differed in composition, diversity, abundance or evenness across the land types. The Type II methanotrophs, however, showed a significantly lower relative abundance in pasture, as well as significant compositional differences across forest and pasture soils (Bray-Curtis $R^2=0.65$, $P < 0.01$, Canberra $R^2=0.18$, $P < 0.05$). The Type II methanotrophs also exhibited significantly higher Shannon diversity and evenness in pasture, relative to forest. The two most abundant Type II genera (*Methylocella* and *Methylosinus*) were the only genera to significantly change in abundance between land types, with both showing a decrease in the pasture. The changes observed in Type II methanotrophs could be of concern because numerous Type II taxa have been shown to be capable of consuming atmospheric levels of methane[40,41] suggesting that these organisms can take up methane even in environments where methane is not produced directly. Other studies, including one in the tropics[42], have reported lower abundance of Type II methanotrophs in agricultural or grassland soils relative to forest soils and have reported decreases in Type II methanotrophs following deforestation[43,44]. For example, in Thailand Knief et al. (2005)[42] observed a lower abundance of Type II methanotrophs (along with a decrease in methane consumption) in agricultural soils relative to tropical forest soils. Thus, the changes we report are in accordance with other reports from agricultural conversion sites and may influence the methane uptake rates of the soils in which they are found.

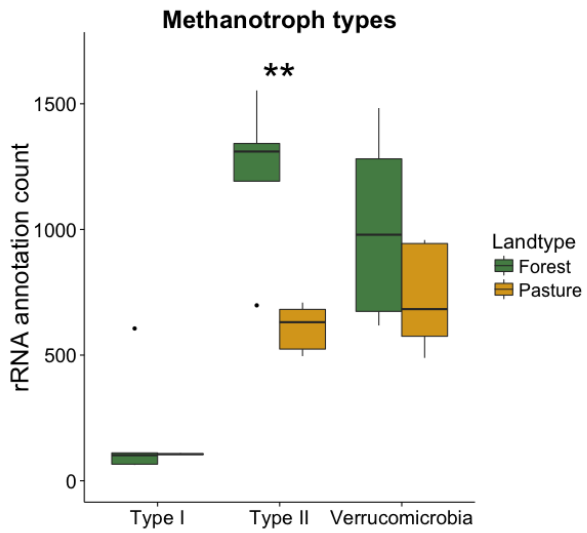

Figure 2: Differential response of methanotroph types to land use change. The abundance of Type I, Type II, and Verrucomicrobia methanotroph rRNA annotations in rainforest and pasture soils. Significant differences between forest and pasture denoted as: ** P < 0.01.

Life history strategies of methanotrophs. We grouped methanotroph genera identified in our study according to the Competitor-Stress Tolerator-Ruderal framework proposed by Ho et al. (2013, see Supplementary Table 3)[22]. We observed that the ratio of Stress Tolerators to Ruderals was significantly lower in the pasture (P<0.001, Fig. 3). This change was driven by a significant decline in the Stress Tolerator group in the pasture (P<0.01) and a marginally significant increase in the Ruderal group (P=0.05) in the pasture. Other strategies characterized as Stress Tolerator (C-S and S-R) were also significantly lower in the pasture (P<0.01) compared to the forest. No other groups were significantly different across the two land types. This could be indicative of a larger trend suggested by other research[5,45] whereby tropical rainforest soils harbor microbial communities that are more stress-tolerant or K-selected (*i.e.* oligotrophic) than that of agricultural soils. By integrating our data into a life history framework we are able to gain a new perspective on the environment as experienced by the microbial community. Much of the differentiation in life history strategies is across the stress gradient. We speculate that the pasture soils may experience stronger fluctuations in temperature, moisture, and oxygen due to increased exposure and decreased light attenuation and hence favor ruderal (disturbance tolerator) life history strategies. By contrast, the forest soil environment, although more heterogeneous over space, may be more stable over time (but overall less rich in nutrients or substrate) and hence favor stress tolerator life history strategies which grow more slowly but tolerate low nutrient conditions. This low substrate environment may also favor strategies exhibiting facultative substrate usage. Many Type II taxa have been shown to be facultative (reviewed in Ho et al. 2013[22]), and this may help to explain why the stress

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tolerator group is significantly higher in forest. Disturbance specialists would likely be able to persist in adverse conditions much like pasture soils, but grow rapidly under periodic shifts to optimal conditions. We see other evidence for this strategy in increases in genes related to dormancy and sporulation ($P < 0.01$, data not shown) and spore DNA protection ($P < 0.001$, data not shown) in pasture soils. These changes to life history traits are likely to influence broader patterns of ecosystem functioning (*e.g.* C cycling) and may serve as a means to assess ecosystem changes, much like a bioindicator, to inform management practices.

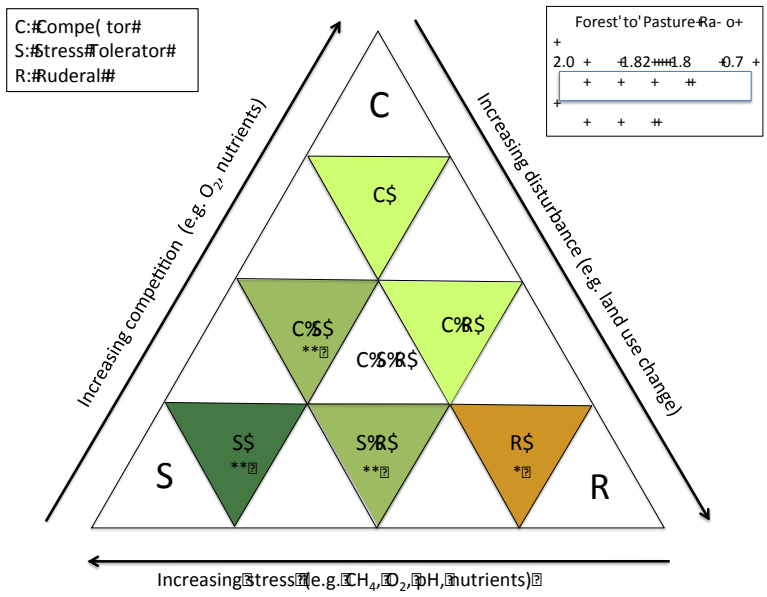

Figure 3: Methanotroph life history strategies vary across rainforest and pasture soils. Life history strategies of methanotroph genera were categorized along three axes (Competitor, Stress tolerator, Ruderal, or combinations thereof) according to the recommendations of Ho et al. (2013)[22]. Triangles are color-coded by the forest-to-pasture abundance ratio of that strategy group. Significant differences between forest and pasture are denoted as follows: ** $P < 0.01$, * $P = 0.05$.

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Methanogenic taxa. The abundance of methanogenic microorganisms did not significantly differ across land types. There were, however, compositional differences between forest and pasture soils. The communities were significantly different in composition across land types (Bray-Curtis R^2=0.61, P<0.001, Canberra R^2=0.31, P<0.01). The average pairwise

dissimilarity was higher in the forest soils than the pasture soils (similar to that observed for the methanotroph community), indicating more variation in community membership in the forest than the pasture. We observed no significant differences in diversity or evenness across sites. The ratio of Acetoclastic-to-Hydrogenotrophic methanogens was significantly higher in the pasture than the forest ($P<0.01$, Supplementary Fig. 1a), driven by a slight (but not significant) increase of acetoclasts and a slight (but not significant) decrease of hydrogenotrophs. When considered separately, the diversity patterns of these two functional groups also varied across forest and pasture. Both groups differed significantly in composition across land types (Acetoclast: Euclidean $R^2=0.30$, $P<0.01$, Hydrogenotroph: Bray $R^2=0.64$, $P<0.05$). However, changes in diversity across land types were not consistent between the acetoclast and hydrogenotroph communities. The acetoclasts had a significantly higher richness in the pasture ($P<0.05$), while the hydrogenotroph community had a significantly lower species richness ($P<0.05$), Simpson diversity ($P<0.01$), Shannon diversity ($P<0.05$), and evenness ($P<0.05$) in the pasture. Six of the eight acetoclast taxa were unique to the pasture. Within the 5 orders of hydrogenotrophs there was also a differential response to land use change. The second most abundant hydrogenotrophic order in the forest (*Methanopyrales*) was completely absent in pasture, while the most abundant hydrogenotrophic order in the pasture (*Methanocellales*) was more than an order of magnitude less abundant in the forest. This differential response could be the result of variable life history strategies within this functional group and could be an interesting avenue for future research.

Changes in gene content

We constructed gene content matrices for genes involved in methanotrophy and methanogenesis (as well as related processes such as homoacetogenesis) using the gene annotations generated by the M5NR database.

Methanotrophy genes. To investigate the potential for methane oxidation in forest and pasture soils, we analyzed differences in the abundance of genes that code for the methane monooxygenase enzyme, as well as genetic markers for Type I and Type II methanotrophs. The methane monooxygenase enzyme is unique to methanotrophs and is the only currently known way that aerobic methanotrophs can utilize methane-derived carbon[21,46]. This enzyme has two forms: soluble methane monooxygenase (sMMO) and particulate methane monooxygenase (pMMO)[21]. pMMO has a higher substrate-specificity and cells containing pMMO tend to have a higher methane affinity and thus may play a role in the consumption of atmospheric methane[21]. Interestingly, we observed that the abundance of genes encoding enzymes in the pMMO pathway was significantly lower in pasture soils ($P < 0.01$, Fig. 4a), but that genes for sMMO did not differ in abundance between forest and pasture (Fig. 4b). Particulate methane monooxygenase is a copper-containing metalloenzyme (while soluble methane monooxygenase does not contain copper); thus the decrease in pMMO could be due to changes in available soil copper in response to land use change[47]. However we did not observe significant differences in the copper content of the forest and pasture soils at our site (data not shown).

There are two biochemical pathways for assimilation of carbon from methane by

methanotrophs: the ribulose-monophosphate pathway (used by Type I methanotrophs) or the serine pathway (used by Type II methanotrophs)[21]. Despite observing changes to the relative abundance and composition of Type II methanotrophs, we did not detect changes in the abundance of genes involved in the ribulose-monophosphate or serine pathways. We attribute these results to the more broad involvement of these pathways in one-carbon metabolism (methylotrophy), being shared by more groups than just methanotrophs[46].

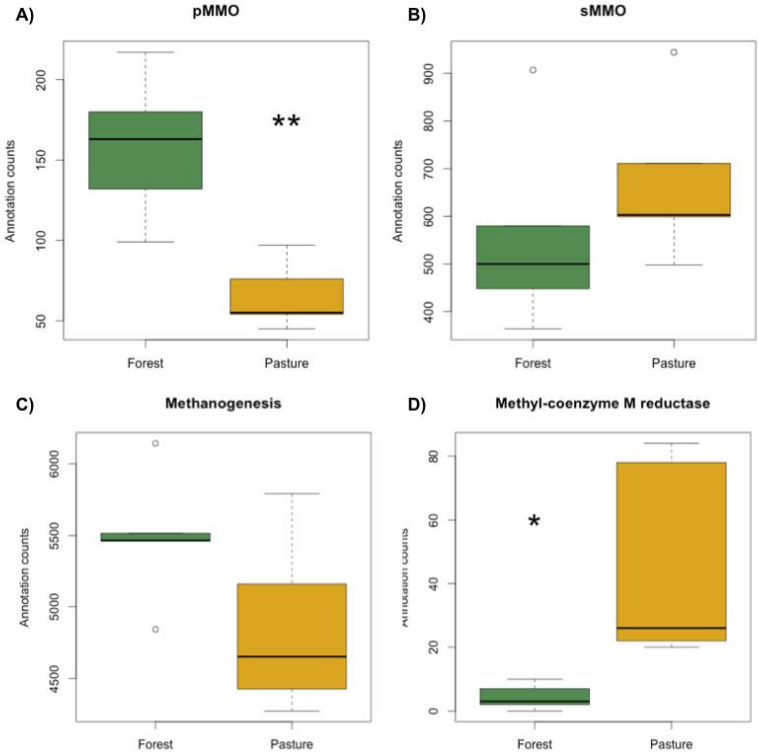

Figure 4: Functional genes related to methane cycling respond differently to land use change: A) The abundance of genes encoding particulate methane monooxygenase (pMMO). B) The abundance of genes encoding soluble methane monooxygenase (sMMO). C) The abundance of genes involved in the methanogenesis pathway. D) The abundance of genes encoding the methyl-coenzyme M reductase enzyme. Significant differences between forest and pasture are denoted as follows: ** $P < 0.01$, * $P < 0.05$.

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Methanogenesis genes. The overall abundance of genes related to methanogenesis (level 3 category), did not significantly differ between land types (Fig. 4c). However, genes encoding the common marker enzyme methyl coenzyme M reductase were significantly higher in the pasture than the forest ($P < 0.05$, Fig. 4d). This could result from changes in gene copy number due to compositional shifts in the community, or it could mean that this

marker increased while other markers in the methanogenesis pathway decreased (and hence the abundance of methanogenesis genes as a whole did not change). Genes involved in methanogenesis from methylated compounds (an alternative pathway by which methane can be produced) were also significantly more abundant in the pasture ($P < 0.05$, Supplementary Fig. 1b). This pathway has not been the focus of very many landscape-level studies, and it is thus unclear to what extent these changes might influence soil methane emissions. Finally, we also observed that genes encoding two enzymes involved in the production of acetate (formyltetrahydrofolate synthetase and acetyl-CoA synthase (subunit beta)) are significantly more abundant in the pasture ($P=0.05$, $P<0.05$, respectively). This indicates that the potential to synthesize acetate (a prerequisite for acetoclastic methanogenesis) is higher in pasture and could be related to the increase in the acetoclastic-to-hydrogenotrophic methanogen ratio we observed in the pasture soils.

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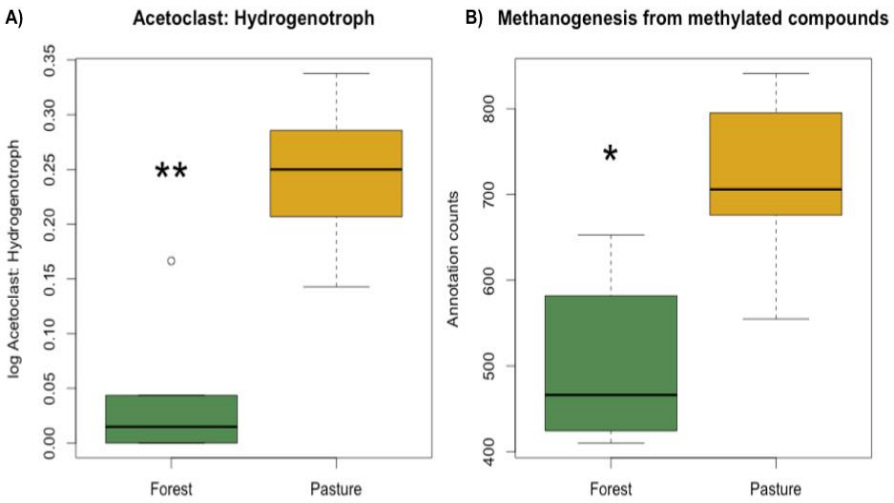
Previous research has shown that forest soils are methane sinks while agricultural (including pasture) soils are methane sources[17,43] . This is true of tropical soils, including soils in the Amazon basin [9,12,13], and specifically those at the site at which our study was conducted[10]. The causes of this difference remain unknown. Researchers have proposed that increased water filled pore space (%WFPS) and decreased O_2 diffusion in pasture and agricultural soils are responsible for this trend, through reduction in oxygen and a subsequent increase in methanogenesis (a strictly anaerobic process)[9]. However, other studies have correlated increases in methane fluxes with changes in methanotroph community traits (e.g. community structure, diversity, and activity[16–18,48]). Evidence from our study suggests that changes to the methane-cycling community could be playing a role in the observed shift from methane sink to source[10] at our site. Although we observed some differences in the methanogen communities of forest and pasture (most notably a change in the relative abundance of acetoclasts), there were few large-scale shifts to the methanogen community (e.g. the abundance of methanogens did not change across sites, nor did the abundance of total genes involved in methanogenesis pathways). In general we observed much larger and more varied differences between forest and pasture for the methanotroph community. Among the most striking differences we observed were a decrease in the abundance of methanotroph taxa, a decline in the methanotroph-to-methanogen ratio, a decrease in pMMO abundance, a decrease in the relative abundance of Type II methanotrophs, and a decrease in the methanotroph Stress Tolerator-to-Ruderal ratio. Hence the shift to methane emission at our site may be due, at least in part, to altered methane consumption rates caused by changes to methanotroph community traits.

Our work illustrates the importance of using environmental metagenomics approaches to address questions regarding microbial functional ecology. The results we report could not have been obtained without an in-depth use of metagenomics. Our gene annotations yielded a wide breadth of genes; 13,418 different genes, 245 of which have the potential to be directly involved in the cycling of methane. The depth at which we performed sequencing allowed us to investigate changes to rare genetic traits that are difficult to assess without PCR amplification (*i.e.* those involved in methanotrophy or acetate production). We were able to detect methanotrophic taxa (*e.g. Methylocella*, which is not known to have pMMO[49]) that would not have been detected using PCR-based, culture independent methods (i.e. amplification of pmoA gene regions). The use of metagenomics also allowed us to simultaneously survey taxa and genes across multiple functional groups. Finally, our work is an example of the power of combining trait inference from

metagenomics with life history theory to generate novel hypotheses regarding the functional responses of microorganisms to environmental change[26].

Although a variety of studies have investigated the impact of land use change on methane cycling organisms, to our knowledge this is the first to do so in the Amazon Basin and to do so using metagenomics. The majority of past studies have focused on temperate regions where rates of agricultural conversion have been relatively low over the last 50 years. Tropical regions, by contrast, are currently facing a faster rate of land use change than any other region[1–3], and the Amazon Basin is facing the highest rate of all tropical regions. Numerous studies have reported increases in methane flux from soil in the Amazon as a result of agricultural development[9,10,12,13]. Here, we present evidence that there are numerous alterations to methane-cycling community traits and that the majority of these traits have been linked to variation in methane flux in other studies[16–18,48]. Thus we suggest that alterations to soil microbial communities could be one of the driving factors behind the shift from methane sink to source following land use change[10] .

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Land use change impacts methanogen community composition and alternative pathway abundance. A) The ratio of acetoclastic-to-hydrogenotrophic methanogens is higher in pasture than forest soils. B) The abundance of genes related to methanogenesis from methylated compounds is higher in pasture relative to forest soils. Significant differences between forest and pasture are denoted as follows: ** $P < 0.01$, * $P < 0.05$.

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