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Structure, function and resilience to desiccation of methanogenic microbial communities in temporarily inundated soils of the Amazon rainforest (Cunia Reserve, Rondonia)

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Originality-Significance Statement:

The Amazonian floodplain is an important source of the greenhouse gas methane. Microbial CH_4 production usually occurs when organic matter is degraded under anaerobic conditions, for example when soils in the rainforest are flooded. The CH_4 is then produced by a complex microbial community consisting of hydrolytic, fermenting Bacteria and methanogenic Archaea. However, a systematic survey with respect to the flooding situation is still lacking. Therefore, we studied rainforest soils with different inundation regimes, including non-flooded soil, occasionally flooded soils, long time flooded soils, and sediments from small forest streams. The potential and resilience of the CH_4 production process were studied in the original soil samples upon anaerobic incubation and again after artificial desiccation and rewetting. We found that the composition and diversity of bacterial and archaeal communities changed systematically in soils from the most dry to the most wet sites. The microbial communities changed relatively strongly, when the soils were desiccated and then incubated a second time under rewetted conditions. Such treatment generally resulted in increased relative abundance of *Firmicutes, Methanocellales* and *Methanosarcinaceae*. Experimental desiccation apparently had a more dramatic effect than the wet/dry field conditions at the different sites, where inundation and drainage changed on an annual rhythm. However, the potential methanogenic activities (rates, pathways) were generally quite similar indicating a rather robust functional resilience against a decrease in microbial diversity.

Abstract

The floodplain of the Amazon River is a large source for the greenhouse gas methane, but the soil microbial communities and processes involved are little known. We studied the structure and function of the methanogenic microbial communities in soils across different inundation regimes in the Cunia Reserve, encompassing non-flooded forest soil (dry forest), occasionally flooded Igapo soils (dry Igapo), long time flooded Igapo soils (wet Igapo), and sediments from Igarape streams (Igarape). We also investigated a Transect (4 sites) from the water shoreline into the dry forest. The potential and resilience of the CH_4 production process were studied in the original soil samples upon anaerobic incubation and again after artificial desiccation and rewetting. Bacterial and archaeal 16S rRNA genes and methanogenic *mcrA* were always present in the soils, except in dry forest soils where *mcrA* increased only upon anaerobic incubation. NMDS analysis showed a clear effect of desiccation and rewetting treatments on both bacterial and archaeal communities. However, the effects of the different sites were less pronounced, with the exception of Igarape. After anaerobic incubation, methanogenic taxa became more abundant among the Archaea, while there was only little change among the Bacteria. Contribution of hydrogenotrophic methanogenesis was usually around 40%. After desiccation and rewetting, we found that *Firmicutes, Methanocellales* and *Methanosarcinaceae* become the dominant taxa, but rates and pathways of CH_4 production stayed similar. Such change was also observed in soils from the Transects. The results indicate that microbial community structures of Amazonian soils will in general be strongly affected by flooding and drainage events, while differences between specific field sites will be comparatively minor.

Introduction

Wetlands are among the largest sources for the greenhouse gas CH_4, emitting annually about 160-210 Tg of CH_4 into the atmosphere (Kirschke et al., 2013). The recent rise in the atmospheric CH_4 budget may be caused by increasing emission from wetlands, tropical wetlands in particular (Nisbet et al., 2016; Schaefer et al., 2016). The Amazon floodplain is a significant CH_4 source to the atmosphere (Crill et al., 1988; Engle and Melack, 2000; Sawakuchi et al., 2014). Inverse modeling of CH_4 concentrations indicate that the Amazon basin contributes up to 7% to the global CH_4 budget (Wilson et al., 2016), being consistent with earlier satellite imagery (Frankenberg et al., 2005). Field studies assessing CH_4 emission also showed that substantial fluxes arise from tropical wetlands, including early studies on wetland surfaces (Bartlett et al., 1988; Devol et al., 1990; Sawakuchi et al., 2014), but also more recent studies on tank bromeliads (Martinson et al., 2010) and floodplain trees (Pangala et al., 2017). The emission of CH_4 by floodplain trees may account for up to 20 Tg per year (Pangala et al., 2017), and is due to production in wetland soil and subsequent escape by tree ventilation (Pangala et al., 2013). Hence, CH_4 production in soils is probably the main driving force for CH_4 emission from tropical wetlands. It is well known that CH_4 production in flooded soils is achieved by methanogenic microbial communities, as shown by numerous studies in wetland soils, rice field soils in particular (Asakawa and Kimura, 2008; Conrad, 2007; Kim and Liesack, 2015; Lueders and Friedrich, 2000; Reim et al., 2017; Rui et al., 2009). In tropical wetlands, however, knowledge is comparatively poor.

Microbial CH_4 production usually occurs when organic matter is degraded under anaerobic conditions, especially after the depletion of oxygen and other electron acceptors with higher energy yield. Such conditions usually occur sometime after flooding, the lag time period depending on the relative availability of degradable organic matter relative to inorganic electron acceptors (Yao et al., 1999). Then, CH_4 is produced by a microbial community consisting of hydrolytic, fermenting and methanogenic microbes. The hydrolytic and fermenting microbes, usually Bacteria, degrade organic matter to acetate, H_2 and CO_2, which are then converted by acetoclastic and hydrogenotrophic archaea to CH_4 (Conrad, 2007). In soil environments, in contrast to saline environments, methylotrophic methanogenesis is

believed to be of negligible importance (Conrad, 2019). Methanogenic archaea typically operate in the absence of oxygen, but nevertheless often can survive in aerated and desiccated soils (Fetzer et al., 1993). Probably it is mainly this plasticity, which allows for the potential of methanogenic functioning in a large variety of different environments, including desert soils (Angel et al., 2011; Peters and Conrad, 1996). Nevertheless, the abundance and composition of the various methanogenic communities can be quite different, for example when comparing methanogenic communities in permanently flooded lake sediments (Ji et al., 2016), seasonally flooded rice field soils (Fernandez Scavino et al., 2013; Reim et al., 2017) or rarely flooded soils (Angel et al., 2012; Hernandez et al., 2017).

Tropical floodplain soils are seasonally flooded and potentially produce CH_4. Such soils exist for example in the Brazilian Pantanal, which causes substantial CH_4 emission (Bastviken et al., 2010; Bergier et al., 2015; Marani and Alvala, 2007). Methane production in Pantanal soil was found to be correlated to the content of organic matter and the number of methanogenic archaea, which consisted of hydrogenotrophic as well as acetoclastic methanogens (Conrad et al., 2011). Methanogenic microbial communities have been studied in sediments of Amazonian oxbow lakes showing differences according to water type (clear, white, and black water) and effects of desiccation and flooding (Conrad et al., 2014b; Ji et al., 2016). However, a systematic survey of the soils in rainforests with respect to the flooding situation is still lacking.

The Amazon rainforest has a well-defined annual water level variation pattern, which regulates the distribution of plant and animal communities according to the intensity, duration and frequency of the flood (Sioli, 1984). This annual variation of the water level between high, decreasing, dry and rising phases modulates the nutrient cycling, mainly carbon (C) and nitrogen (N), and their biogeochemical processes (Moreira-Turcq et al., 2003). In the Amazon region, periodically flooded soils occupy a large area of around 800,000 km^2 (Hess et al., 2015), and cover rivers with different types of water that, because of their physicochemical characteristics, may influence the production of greenhouse gases like CH_4. In addition, ecosystems under the influence of flood pulse present the formation of different types of forest, due to the variation of the water level. The upland forest (*terra firme*) comprises the

great majority of the Amazonian forest area and is located in higher regions, which are not subject to floods, while the lowland forest (Igapó) are periodically flooded during high waters. The lowland vegetation has morphological and physiological adaptations to deal with temporary flooding (Junk et al., 2011).

In this study, we sampled soils from (1) non-flooded forest (dry forest), (2) Igapó that was dry at the time of sampling and get occasionally flooded (dry Igapo), (3) Igapó that was flooded at the time of sampling (wet Igapo), (4) sediments from small streams (Igarapé) that drain water from the forest to larger rivers, and (5) a transect within the Igapó forest from the margin of the water in direction to the non-flooded forest. Our objective was to see to which extent the bacterial and archaeal communities and their methanogenic functioning differed between the different sampling sites and how they reacted upon artificial desiccation and reflooding.

Results

Functional analysis

Methane production activities differed between Dry Forest, Dry Igapo, Wet Igapo and Igarape (Fig. 1). Dry Forest showed the longest lag phase (about 80 days) until CH_4 production started, followed by Dry Igapo (about 20 days), while at the inundated sites (Wet Igapo, Igarape) CH_4 production was almost instantaneous (Fig. 1A). The CH_4 production rates following the lag phase were lowest in Dry Forest (about 3 nmol h^{-1} g^{-1}) and showed higher and similar values (7-13 nmol h^{-1} g^{-1}) at the other sites (Fig. 1B). The contribution of hydrogenotrophic methanogenesis was lowest (<10%) in Dry forest and showed higher and similar values (40-60%) at the other sites (Fig. 1C). The CH_4 production activities were not correlated ($R^2 < 0.1$) with the contents of organic matter (1-5%) and nitrogen (0.1-0.4%) (Fig. 1D) or the pH (5.3-6.5) (Fig. 1E), but were correlated ($R^2 = 0.61$) with the content of total iron (15-18 µmol g^{-1}) (Fig. 1F). Desiccation and rewetting resulted in similar lag phases in all the different site samples (Fig. 1A), while CH_4 production rates did not change much (except an increase in Wet Igapo) after rewetting (Fig. 1B). After desiccation and rewetting CH_4 production rates were correlated with organic C ($R^2 = 0.62$), total iron ($R^2 = 0.48$), and weakly

with pH (R^2 = 0.28). Contribution of hydrogenotrophic methanogenesis increased after desiccation and rewetting in Dry Forest, and decreased in Igarape, but the percentage contribution was generally between 20% and 45% (Fig. 1C).

The Transect was on dry forest land in different distance to a stream (Fig. 2). The lag phase was lowest (25 days) directly at the shoreline (Transect 0) and longer (45-60 days) in larger distance (Fig. 2A). Lag phases decreased upon desiccation and rewetting (Fig. 2A), similarly as at the Dry Forest site and the Dry Igapo sites (Fig. 1A). Methane production rates in the Transect were relatively low (3-6 nmol h^{-1} g^{-1}) and stayed at these values (except a decrease in Transect 2) upon desiccation and rewetting (Fig. 2B). Contribution of hydrogenotrophic methanogenesis decreased from 35% to 20% and increased again to 40% and 65% from Transect 0 to Transect 3 (Fig. 2C). After desiccation and rewetting percentage contribution was still in a range of 20-40% (Fig. 2C). The contents of organic matter (0.7-1.2%), total nitrogen (0.07-0.12%) and total iron (6-12 µmol g^{-1}) were relatively low, the lowest values always at the shoreline (Fig. 2D, 2F), while the pH (5.5-6.2) was similar (Fig. 2E) as at the sites shown in Fig. 1. There was a strong correlation of CH_4 production rates with pH (R^2 = 0.89), but only weak correlations with organic C (R^2 = 0.5) and total iron (R^2 = 0.24). After desiccation these correlations became negligible (R^2 < 0.1) except with total iron (R^2 = 0.17).

Microbial abundance

Copy numbers of bacterial 16S rRNA genes were generally more numerous than those of archaeal 16S rRNA genes and of *mcrA* genes, both at the different sites (Fig. 3) and the Transect (Fig. S1). Copy numbers of bacterial and archaeal 16S rRNA genes changed slightly or strongly, respectively, with incubation, desiccation and rewetting. Usually, numbers increased after incubation, decreased after desiccation, and increased again after rewetting. However, these changes were usually within one order of magnitude. By contrast, there were only minor differences between the different sites (Fig. 3) and within the Transect (Fig. S1). This was also the case for the copy numbers of *mcrA* representing the methanogenic archaea, but with the exception of the sites Dry Forest. In Dry Forest, *mcrA* copy numbers were originally very low (<10^6 copies g^{-1}) and only increased upon anaerobic incubation (Fig. 3).

However, all the other sites contained relatively high numbers (around 10^8 g^{-1}) of *mcrA* copies right from the beginning.

Bacterial community composition

The bacterial communities at the different sites (Fig. 4) and the Transect (Fig. S2) consisted of several major phyla (>5% relative abundance) including *Acidobacteria, Actinobacteria, Chloroflexi, Firmicutes, Planctomycetes, Proteobacteria,* and *Verrucomicrobia*. The Shannon diversity indices were originally between 6.7 and 7.9 (Fig. 5) (species evenness see Table S1). In general, diversity was only slightly larger at Igarape than at the other (Dry Forest, Dry Igapo, Wet Igapo, Transect), but always decreased upon desiccation and did not completely recover upon rewetting (Fig. 5). Composition of bacterial communities changed systematically along the Transect (Fig. S2). Non-metric multidimensional scaling (NMDS) analysis showed that the composition of the bacterial communities systematically also changed across soils from Dry Forest, Transect, Dry Igapo, Wet Igapo, to Igarape (Fig. 6). Although composition changed relatively little during the first anaerobic incubation before desiccation (I, O), it changed a lot after desiccation (D, R) (Fig. 6). Key species reflecting this dynamics were found among the *Acidobacteria, Actinobacteria, Alphaproteobacteria, Chloroflexi* and *Firmicutes* (Fig. 6). It was the phylum *Firmicutes*, which generally increased most in relative abundance (reaching >30%) when soils were desiccated and rewetted (Fig. 4, Fig. S2). Interestingly, desiccation alone mainly resulted in increase of the phyla *Chloroflexi* (mainly *Ktedonobacteria*), *Actinobacteria* and *Firmicutes,* whose DNA seemed to be most resistant, whereas anaerobic incubation upon rewetting greatly stimulated the *Firmicutes*.

Archaeal community composition

The archaeal communities at the different sites (Fig. 4) and the Transect (Fig. S2) consisted at all sites mainly of the (non-methanogenic) class *Thaumarchaeota*, except at Igarape where *Methanobacteriales, Methanocellales, Methanomicrobiales, Methanosaetaceae* (now renamed *Methanotrichaceae* (Oren, 2014)), *Methanosarcinaceae, and Methanomassiliicoccus*

accounted for >30% of the total archaeal abundance (Fig. 4). Dry and Wet Igapo also showed these methanogenic taxa albeit only at <5% relative abundance. NMDS analysis showed that the composition of the archaeal communities (similar as the bacterial communities) systematically changed across soils from Dry Forest, Transect, Dry Igapo, Wet Igapo, to Igarape (Fig. 6). At all sites the relative abundance of putatively methanogenic archaea consecutively increased relative to those of non-methanogenic archaea, mostly *Thaumarchaeota*, when the soils were anaerobically incubated, desiccated and rewetted. In the end, the most abundant methanogens always belonged to the *Methanocellales*, *Methanosarcinaceae* and *Methanomassiliicoccus* (in Wet Igapo also to Rice_Cluster_II) (Fig. 4, Fig. S2). The Shannon diversity indices were originally between 2.9 and 4.7 and highest at Igarape (Fig. 5) (species evenness see Table S1). In the end of the incubation series the diversity was always (except Dry Forest) smaller than in the beginning (Fig. 5). This change in community composition was also shown by NMDS analysis, in which the desiccated and rewetted samples clustered together, showing *Methanocella*, *Methanosarcina* and *Methanomasilliicoccus* among the key genera (Fig. 6). NMDS also showed that the archaeal communities were different in the Igarape compared to the other sites (Fig. 6).

Discussion

Function of the soil methanogenic communities

Our study showed that virtually all different types of soils from the Amazon rainforest had the capacity to produce CH_4, provided conditions were wet and anaerobic. All soils had microbial communities that were able to produce CH_4 under such conditions indicating that CH_4 production indeed did happen before and will happen again in future. Thus, it explains why the Amazon forest provides for a substantial source of the greenhouse gas CH_4 (Pangala et al., 2017). However, the different Amazonian soils had different propensities for CH_4 production, which were highest in soils that were flooded at the time of sampling and lowest in soils that had presumably not been flooded for a long time. The propensity for CH_4 production was best seen in the duration of the lag phase.

In Amazon forest soils with different histories of inundation CH_4 production started after different lag phase periods. These were longest for sites that were never flooded (i.e., Dry Forest), were negligible in permanently flooded sites (i.e. Igarape), and were in-between at the other sites (Dry and Wet Igapo, Transect). This observation was striking and was to some extent paralleled by the potential rates of CH_4 production, which were relatively lower in soils from the permanently dry sites. The reason for these differences was probably whether iron was present in oxidized versus reduced form and whether microbial activity, methanogenic activity in particular, was potentially available. It is well known that CH_4 production only starts when the available Fe(III) has been reduced to Fe(II), being the time depending on the availability of electron donors (usually from the degradation of organic matter), the temperature and the microbial activity (Ginn et al., 2014; Yao et al., 1999; Yao and Conrad, 2000a). Potential CH_4 production rates were indeed correlated to some extent to the contents of organic C and total iron, similarly as observed before in sediments from Pantanal lakes (Conrad et al., 2011). Such correlation is consistent with the observation in rice field soils that rates and amounts of CH_4 production increase with the ratio of available electron donors to electron acceptors, e.g. organic carbon to ferric iron (Yao et al., 1999). The total iron content of the soils was at the lower end of a range found in various paddy soils and lake sediments (Fageria et al., 2008; Fernandez Scavino et al., 2013; Hernandez et al., 2017; VanBodegom et al., 2003; Wissing et al., 2014; Yao et al., 1999) including those from the Brazilian Pantanal (Conrad et al., 2011) or in Amazonian oxbow lakes (Ji et al., 2016). The content of organic carbon was also similar to that found in paddy soils. Since contents of iron and organic carbon were in a similar range in all the different soils, it is reasonable to assume that the ratio of Fe(III) to Fe(II) was related to the extent and history of inundation, meaning that it was higher at non-flooded than at flooded sites.

However, this explanation is not sufficient, since lag periods decreased strongly after artificial desiccation and rewetting, although after such treatment the ratio of Fe(III)/Fe(II) should be maximal. We assume that microbial activity had been limiting in the beginning, increased during the first incubation, and largely survived the desiccation treatment, so that CH_4 production could then start much earlier. This conclusion is supported by the observation

that numbers of methanogenic archaea (measured as copies of *mcrA*) were indeed relatively low in soils from the dry sites (Dry Forest, Transect), increased during the first incubation and were not severely reduced after desiccation. This is well seen from the data of the Transect, where numbers of methanogens were initially lower at the sites that were more distant from the water margin. Similar observations have been made with flooded rice field soils and lake sediments, which maintain high numbers of methanogens after desiccation, while dry upland soils generally contain very low numbers of methanogens, which only increase upon prolonged flooding (Angel et al., 2012; Peters and Conrad, 1996). We therefore hypothesize that the numbers of methanogens is an indication for history of flooding, in a way that methanogenic populations will survive relatively short periods of desiccation, while they will die after long periods of non-flooded conditions. However, the time limits for survival and death are presently not clear, and also whether and how such time limits depend on the physicochemical soil conditions. Besides such rather conventional explanations, it might also be possible that recently discovered microbial interactions, such as direct interspecies electron transfer (Lovley 2017; Shi et al., 2016), were involved in the initiation and maintenance of CH_4 production. However, such role is pure speculation at the present stage of investigation.

The pathway of CH_4 production was for all sites in a similar range of <40% contribution by hydrogenotrophic methanogenesis, which is characteristic for situations in which polysaccharides are completely degraded to acetate and H_2/CO_2, which then contribute in a ratio of >2/3 to <1/3 to total CH_4 production (Conrad, 1999). This situation seems to be almost generally the case in flooded paddy soils (Hernandez et al., 2017; Reim et al., 2017; Yao and Conrad, 2000b). An increased contribution of hydrogenotrophic methanogenesis has been proposed to indicate that organic matter is relatively resistant to degradation, such as in deep lake sediments and peat, but also in soil upon long periods of anaerobic degradation without supply of fresh material (Conrad et al., 2011; Hodgkins et al., 2014; Ji et al., 2018; Liu et al., 2017). Hence, the relatively large contribution of hydrogenotrophic methanogenesis in Wet Igapo and one site of the Transect may indicate that the soil organic matter was relatively hard to degrade, a conclusion that is supported by the comparatively lower rates of CH_4 production at these sites.

Structures of the methanogenic microbial communities

The bacterial and the archaeal communities in the different soils had a size (about 10^7-10^9 16S rRNA gene copies g^{-1}) that is typical for soils in general. The bacterial numbers were usually higher than the archaeal numbers, which is also a common observation. However, copy numbers of methanogenic *mcrA* were initially very low in the Dry Forest and in the Transect. The archaeal communities at these sites consisted almost entirely of non-methanogenic *Thaumarchaeota*, which may have a function in archaeal ammonia oxidation (Spang et al., 2010). The relative abundance of methanogenic taxa only increased after anaerobic incubation. Then all the soils contained methanogenic taxa at a relative abundance of at least 10%, and also exhibited high numbers of *mcrA* copies. The bacterial community structures before and right after the incubation were similar. The microbial community structures allowed CH_4 production in all the different soils. Hence, the community structures were not necessarily limiting for CH_4 production, as for example, indicated by the lower CH_4 production rates in Igarape sediments than in Wet Igapo soils, despite the fact that the relative abundance and diversity of methanogenic taxa were much larger in the former than in the latter. The Igarape sediments had the highest bacterial and archaeal diversities, and the potentially methanogenic archaeal taxa had the largest relative abundance (>50%) among all the different sites.

Because the bacterial communities changed only little by the anaerobic incubation, the observed lag phase cannot be pinned to particular bacterial phyla. Only in the Dry Forest and the Transect soil, relative abundance of *Deltaproteobacteria* increased, perhaps since iron reducers, which are common in this class, proliferated during the lag phase reducing still available Fe(III). Also *Firmicutes* slightly increased in soils from the dry sites maybe allowing more fermentation, while in sediments of the Igarape sites, *Bacteroidetes* (also potentially involved in fermentation) increased. The minor change in bacterial community structure during the first anaerobic incubation was also seen in NMDS analysis by the close clustering of bacterial OTUs. NMDS analysis also showed that the composition of the bacterial communities displayed a slight but systematic change from the most frequently dry

sites (Dry Forest) to the most frequently inundated sites (Igarape) showing that inundation history did affect microbial community composition.

However, both bacterial and archaeal community compositions changed dramatically when the soils were desiccated and rewetted. Desiccation alone already had a strong effect, and the rewetting with subsequent anaerobic incubation again changed the composition of the microbial communities. After desiccation, the bacterial communities in the dry soils generally decreased in diversity with *Chloroflexi (Ktedonobacteria)*, *Actinobacteria* and *Firmicutes* increasing in relative abundance. All these bacteria are known to produce resting stages, which, we think, made them (or their DNA) relatively resistant to desiccation. After rewetting and second incubation, however, only *Firmicutes* remained as a dominant (>30% relative abundance) phylum, probably reflecting their role in anaerobic fermentation. The eventual dominance of *Firmicutes* seems to be a general feature when flooded soils or sediments are artificially desiccated and rewetted (Angel and Conrad, 2013; Conrad et al., 2014b; Hernandez et al., 2017; Ji et al., 2015; Reim et al., 2017).

Among the Archaea, desiccation alone drastically decreased the relative abundance of *Thaumarchaeota*, mainly favouring *Methanocellales* and *Methanosarcinaceae*, but also *Methanomassiliicoccus*, Rice_cluster_II (only Igarape), *Methanobacteriales* (only Transect 3) and miscellaneous Crenarchaeota (MCG). Rice_Cluster_II contains the methanogenic *Candidatus* 'Methanoflorens stordalenmirensis' (Mondav et al., 2014). Members of MCG may also have the potential for CH_4 formation (Evans et al., 2015; Spang et al., 2017). Hence, desiccation resulted in increase of potentially methanogenic archaea in favor of non-methanogenic *Thaumarchaeota*. Rewetting and second incubation manifested this situation resulting in dominance of mainly *Methanocellales* and *Methanosarcinaceae*. Such dominance seems to be a general feature when flooded soils or sediments are artificially desiccated and rewetted (Conrad et al., 2014b; Hernandez et al., 2017; Ji et al., 2015; Reim et al., 2017). It may be due to the antioxidant features of these methanogenic taxa (Erkel et al., 2006; Lyu and Lu, 2018).

Although the desiccation-rewetting cycle resulted in a decrease of the diversity of both Bacteria and Archaea, the methanogenic degradation of organic matter was nevertheless fully

functional. Soils dominated by *Firmicutes, Methanocellales* and *Methanosarcinaceae* were apparently able to operate similarly (similar rate and pathway of methanogenesis) as when the soils contained a balanced mixture of various bacterial and archaeal taxa. Similar observations have been made before testing a variety of different soils and sediments (Angel et al., 2012; Conrad et al., 2014b; Hernandez et al., 2017; Ji et al., 2015; Reim et al., 2017). It was expected that desiccation resulted in a loss of sensitive microbial species and thus in a decrease of diversity. It is noteworthy that this loss was not recovered by rewetting and anaerobic incubation within a period of more than a month. We assume that this inability for recovery of community structure was due to our closed incubation system, which did not allow invasion and recolonization of the impoverished soil. In nature by contrast, recolonization should be possible after phases of dryness. In rice fields that are rotated between flooded rice and upland crops, the archaeal communities were dominated by methanogenic *Euryarchaeota* versus non-methanogenic *Thaumarchaeota*, respectively (Breidenbach et al., 2017).

In the different Amazon rain forest soils the Igarape sediments clearly clustered separately from all the other soils in NMDS analysis. Nevertheless, there was also a visible gradient in community composition from Dry forest soil, Transect, Dray Iago to Wet Igapo. Although these gradients, which follow the extent and frequency of inundation, are consistent with the effects of artificial desiccation, they by far do not reach the community composition seen after the experimental treatment. We assume that the rainforest soils will rarely experience complete and large-scale desiccation. Together with the possibility for invasion and recolonization, community composition may only gradually shift between wet and dry conditions. Nevertheless, these shifts were apparently sufficient to create different propensities for CH_4 production.

Conclusions

Our study at Cunia Reserve of soils from sites, which had different histories of inundation, showed that the compositions of bacterial and archaeal communities changed systematically in soils from the most dry (Dry Forest) to the most wet (Igarape) sites and also changed across

a transect from the water edge into the dry forest. Nevertheless, the microbial community compositions in all these soils were relatively similar in composition and diversity, with sediments of the Igarape streams being the most dissimilar ones (Fig. 6). By contrast, composition and diversity of microbial communities changed relatively strongly, when the soil or sediment samples were desiccated and then incubated a second time under rewetted conditions (Fig. 6). The desiccation and rewetting treatments generally resulted in increased relative abundance of *Firmicutes, Methanocellales* and *Methanosarcinaceae*. Similar changes had been observed before in soils and sediments from a variety of different wetland ecosystems, so that we may speculate that these bacterial and archaeal taxa are the most tolerant and resilient ones, when anoxic wetland sites are drained and thus exposed to dryness and oxygen. Short-term desiccation apparently had a more dramatic effect on the microbial community compositions than the wet/dry conditions at the different field sites, where inundation and drainage changed on an annual rhythm. However, the potential methanogenic activities (rates, pathways) in the soils before and after the desiccation treatment were quite similar despite the large differences in microbial community composition. Such similarity indicates a rather robust functional resilience against a decreasing microbial diversity with a few taxa of fermenting bacteria (e.g., *Firmicutes*) and hydrogenotrophic (*Methanocellales, Methanosarcinaceae*) and aceticlastic (*Methanosarcinaceae*) methanogens dominating.

Experimental procedures

Sampling sites

The soil samples were taken in the Ecological Station of Cuniã, which is localized in the Madeira River sub basin within the Amazon catchment about 120 km from Porto Velho, Rondônia, Brazil (Fig. 7). In this area we studied four different flooded forest areas (Igapó 1, 2, 3 and 4) and seven different streams (Igarapé) (F1, F2, F3, F4, F5, F6 and F7). Soil samples from 0-10 cm depth were taken in 2013 between May 2 and 4 at the following sites using a corer. The samples were placed in plastic bags (dry soil sites) or in completely filled and stoppered glass bottles (wet soil sites) and transported in styrofoam boxes within two weeks to Marburg, where experiments were initiated immediately.

Dry forest: two plots of primary forest (non flooded areas) were sampled. Both plots were dominated by palm species. The distance between plot 1 and 2 was 2 km. Soil samples were taken at each plot in 5 replicates and transported in zip-lock bags.

Dry Igapó Forest: These are sites that are certainly flooded during some months of the year. However, during the time of sampling the soil was not flooded and already dry. Four plots were sampled, which were all located within 3 m distance from the shore of water. The distances between the plots varied between 0.3 and 1 km. Soil samples were taken at each plot in 5 replicates and transported in zip-lock bags.

Wet Igapó Forest: Three plots from the flooded part of the Igapó forests were sampled. The flooding water was about 1 m deep. Samples were taken without replication, and were only technically replicated (n=3) for laboratory experiments.

Igarapé Sediments: Igarapés are small streams that drain the water from the forest to larger rivers. All sampled Igarapé streams were with water during the whole year. The sediments of six different Igarapés were sampled below about 1 m water depth, close to the shore. Samples were taken without replication, and were only technically replicated (n=3) for laboratory experiments.

Transect: soil samples were taken in a transect from the shore of a stream (Transect spot in Figure 7) in direction to the non-flooded forest. This area was different from the other Igapó areas. Samples were at (i) 0 to 0.5 m distance from the water; (ii) 1 to 1.5 m distance; (iii) 2 to 2.5 m distance; and 3 to 3.5 m distance. At each plot 5 replicate samples were taken.

Incubation conditions

The incubation procedure was as described by Ji et al. (2015). Non-flooded soil samples (Dry Forest, Dry Igapo, Transect) were available each as 5 true replicates. Flooded soil samples (Wet Igapo, Igarape) were available as single samples and thus, were technically replicated (n=3). Soil (7-8 g of Igarape sediment and 5 g for the other environments) placed into a 26-ml glass pressure tube, 5 ml anoxic sterile water was added and the tube was closed with a black rubber stopper. The gas phase of the tubes was exchanged with N_2 (10 times evacuation and regassing). A parallel set of samples was prepared that contained 3%

methylfluoride, an inhibitor for acetoclastic methanogenesis (Janssen and Frenzel, 1997). The tubes were then incubated without shaking at 25°C for about 130-170 days (Dry Forest), 45 days (Dry Igopo), 35-55 days (Wet Igapo, Igarape), and 80-110 days (Transect) until stabile CH_4 production had established. At the end of the incubation, the slurries were dried at 37°C (drainage period) for several weeks until weight was constant. To mimic reflooding, the dried soil was rewetted by addition of 5 ml water and reincubated at 25°C for another 40-60 days.

Chemical analyses

The chemical analyses were done as described before (Conrad et al., 2014a). The following analyses were done on the original soil samples: total iron, pH, organic carbon, and total nitrogen. The $\delta^{13}C$ of organic matter was analyzed by the Centre for Stable Isotope Research and Analysis (KOSI) at the University of Göttingen using an elemental analyzer coupled to an IRMS.

Gases (CH_4, CO_2) were analyzed frequently during the incubation by gas chromatography (GC), and their $\delta^{13}C$ values by combustion isotope ratio mass spectrometry (GC-C-IRMS). Total acetate and its $\delta^{13}C$ was analyzed at the end of incubation by high-pressure liquid chromatography (HPLC) and HPLC-C-IRMS, respectively. The lag time until onset of CH_4 production was defined as the time point at with CH_4 production started. The rate of CH_4 production was determined by linear regression of the period of constant CH_4 production (> 6 time points). The fraction (f_{H2}) of CH_4 production by hydrogenotrophic methanogenesis was calculated by mass balance as described before (Conrad et al., 2010) using

$$f_{H2} = (\delta^{13}C_{CH4} - \delta^{13}C_{CH4\text{-}ma})/(\delta^{13}C_{CH4\text{-}mc} - \delta^{13}C_{CH4\text{-}ma}) \quad (1)$$

with $\delta^{13}C_{CH4}$ = $\delta^{13}C$ of total CH_4 produced, $\delta^{13}C_{CH4\text{-}mc}$ = $\delta^{13}C$ of CH_4 produced from hydrogenotrophic methanogenesis, which is equivalent to the CH_4 produced in the presence of CH_3F, and $\delta^{13}C_{CH4\text{-}ma}$ = $\delta^{13}C$ of CH_4 produced from acetoclastic methanogenesis. The $\delta^{13}C_{CH4\text{-}ma}$ was assumed to be equal to $\delta^{13}C_{ac\text{-}methyl}$, i.e., no fractionation during the reduction of acetate-methyl to CH_4. The $\delta^{13}C$ of total acetate was measured at the end of the incubation in the presence of CH_3F. The $\delta^{13}C$ of the methyl group of acetate was assumed to be 8‰ more negative than that of total acetate (Conrad et al., 2014a).

DNA extraction and qPCR

Soil DNA was extracted from the original soil samples (O), at the end of the incubation (I), after drying of the soil (D), and at the end of reincubation (R) using all three of the technical replicates. Soil DNA was extracted using the NucleoSpin Soil Kit (Macherey-Nagel, Düren, Germany). Lysis buffer SL2 and enhancer SX were used and DNA was eluted in 100µl of Elution Buffer. Extracted DNA was used as template for qPCR and MiSeq Illumina analyses. The abundance of archaeal 16S rRNA and of methanogenic *mcrA* gene was determined by qPCR with primer sets Arch364-f/ 934b-r and mlas-mod-f/mcrA-rev-r respectively (Angel et al., 2012; Kemnitz et al., 2005), and conditions were as follows: for archaeal 16S rRNA gene: 6 min at 94°C, 40 cycles of 94°C for 35 s, 66°C for 30 s, 72°C for 45 s, 86.5°C for 10 s (snapshot) and for *mcrA* gene: 5 min 94°C, 40 cycle at 95°C for 30 s, 57°C for 45 s, 72°C for 30 s, 84°C for 10 s (snapshot). QPCR cycling conditions for bacterial 16S rRNA were as follows: 94°C for 8 min, 50 cycles for 94°C for 20 s, 50°C for 20 s, 72°C for 50 s (snapshot) using the primer set 519-f/907-r (Lane, 1991). Clonal DNA (Angel et al., 2012) was used for qPCR of *mcrA* and archaeal 16S rRNA genes, and genomic DNA (from *E. coli*; Stubner, 2004) for bacterial 16S rRNA genes. For archaeal 16S rRNA genes efficiencies of 87,6 - 88,2% with R^2 values > 0.99 were obtained. For bacterial 16S rRNA genes efficiencies of 74,1% with R^2 value of 0.99 were obtained. For *mcrA* genes efficiencies of 72,5 - 77,8% with R^2 values > 0.99 were obtained. Technical duplicates were performed for each of the replicates.

Illumina library preparation and sequencing

PCR primers (515F, 5'-GTGCCAGCMGCCGCGGTAA-3' and 806R, 5'-GGACTACVSGGGTATCTAAT-3') targeting the V4 region of the 16S rRNA gene (approximately 250 nucleotides) for both archaeal and bacterial were used (Bates et al., 2011). Individual PCRs contained a 6-bp molecular barcode integrated in the forward primer. PCR conditions consisted of an initial denaturation at 94°C for 5 min, followed by 28 cycles of 94°C for 30 s, 50°C for 30 s, and 68°C for 30 s and a final extension at 68°C for 10 min

(Hernandez et al., 2015). Amplicons were purified using a PCR cleanup kit (Sigma) and quantified using a Qubit 2.0 fluorometer (Invitrogen). Finally, samples were pooled in an equimolar concentration and sequenced on separate runs for MiSeq using a 2 x 300 bp paired end protocol. Library preparation and sequencing was performed at the Max Planck Genome Centre (MPGC), Cologne, Germany.

Bioinformatic steps were followed as described previously (Hernandez et al., 2017). Briefly, quality filtering and trimming adaptors were done with cutadapt (Martin, 2011). Merge of the reads was carried out using the usearch fastq_mergepairs command (Edgar, 2013). Operational taxonomic unit (OTU) clustering (97%) and de novo chimera filtering was carried out using UCHIME (Edgar et al., 2011).

Sequence data were deposited in the NCBI Sequence Read Archive (SRA) under accession number PRJNA429349.

Statistical analyses and OTU classification

Whereas the plots from the dry sites were sampled in true replicates, those from the wet sites (Wet Igapo, Igarape) were sampled without replication and were only technically replicated. Therefore, we treated the averages measured in the different plots as replicates of each site, and averaged them for each site, i.e., Dry Forest (n=2), Dry Igapo (n=4), Wet Igapo (n=3), Transect (n=4), and Igarape (n=6). For the Transect, we also compared the different plots, for which true replicates existed (n=5). Illumina sequencing of the Transect sites was done in pooled samples without replication. All statistical analyses were performed using the vegan package (Oksanen et al., 2013) in R software version 3.0.2 (http://www.r-project.org). Tests with $P \leq 0.05$ were considered to be statistically significant. Gene abundances within the soils were compared by one-way analysis of variance (ANOVA) followed by a Tukey post hoc test. ANOVA was also performed between the soils for lag phase, CH_4 production, fraction hydrogenotrophic, organic C, total N, pH and Fe. For all OTU-based statistical analyses, the data set was normalized by a Hellinger transformation (Legendre and Gallagher, 2001) using the *decostand* function. For the alpha-diversity indices, Shannon index (H), Species evenness (J) were carried out using the diversity calculators. Alpha-diversity indices

were calculated based on the lowest number of sequences available from each site, *i.e*., 361789 for bacterial- and 5590 for archaeal-16S rRNA gene reads (subsample using the *rrarefy* function). This procedure standardizes the measures needed for comparison. For beta-diversity, non-metric multidimensional scaling (NMDS) ordination of Hellinger distances was carried out using the *cmdscale* function. The influence of representative OTUs explaining most of the differences between samples were defined as the OTUs contributing the largest absolute loadings in the first and second dimensions of the PCA (Breidenbach et al., 2016), obtained from the rotation output file were included into the NMDS by using the *envfit* function (vegan package in R, permutations = 999).

A representative sequence from each of the OTUs was classified with the mother software platform (Schloss et al., 2009). Sequences were aligned against the SILVA bacteria 16S rRNA gene database using the naïve Bayesian classifier with a bootstrap confidence threshold of 80%.

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Figure legends

Fig. 1: Functional data for soils from different sites with different histories of inundation in Cunia Reserve. The data include (A) lag phases, (B) rates and (C) pathways of CH_4 production potential measured (I) after the initial incubation under anaerobic conditions and (R) after desiccation and rewetting; they further include (D) contents of organic carbon and total nitrogen, (E) pH values, and (F) total iron contents. The error bars are standard errors of n=2-6. Significant differences were tested using one-way analysis of variance with Tukey's post hoc test at $P \leq 0.05$. Different letters above the bars indicate significant differences between sites (lowercase letters for initial anaerobic incubation and capital letters for rewetted incubation and for total N).

Fig. 2: Functional data for soils from the transect in Cunia Reserve. The transect sites were at (0) 0-0.5 m, (1) 1-1.5 m, (2) 2-2.5 m, and (3) 3-3.5 m distance from the water edge. The data include (A) lag phases, (B) rates and (C) pathways of CH_4 production potential measured (I) after the initial incubation under anaerobic conditions and (R) after desiccation and rewetting; they further include (D) contents of organic carbon and total nitrogen, (E) pH values, and (F) total iron contents. The error bars are standard errors of n=5. Significant differences were tested using one-way analysis of variance with Tukey's post hoc test at $P \leq 0.05$. Different letters above the bars indicate significant differences between sites (lowercase letters for initial anaerobic incubation and organic C; capital letters for rewetted incubation, Fe and for total N).

Fig. 3: Copy numbers of (A) bacterial and (B) archaeal 16S rRNA genes and (C) methanogenic *mcrA* for soils from different sites with different histories of inundation in Cunia Reserve, assayed (O) in the original soil, (I) after intital anaerobic incubation, (D) after desiccation, and (R) after rewetting and second incubation. The error bars are standard errors of n=2-6. Significant differences were tested using one-way analysis of variance with Tukey's post hoc test at P

817 ≤ 0.05. Different letters above the bars indicate significant differences between
818 the treatments.
819 Fig. 4: Composition of the (A, B, C, D) bacterial and (E, F, G, H) archaeal
820 communities for soils from different sites with different histories of inundation
821 in Cunia Reserve, i.e., (A, E) Dry Forest, (B, F) Dry Igapo, (C, G) Wet Igapo,
822 and (D, H) Igarape. The community compositions were determined (O) for the
823 original soil, (I) after initial anaerobic incubation, (D) after desiccation, and (R)
824 after rewetting and second incubation.
825 Fig. 5: Diversity (Shannon index) of (A) bacterial and (B) archaeal 16S rRNA
826 gene sequences in soils from different sites with different histories of
827 inundation and from the transect in Cunia Reserve, determined (O) in the
828 original soil, (I) after initial anaerobic incubation, (D) after desiccation, and (R)
829 after rewetting and second incubation. The error bars are standard errors (Dry
830 Forest: n=2; Dry Igapo: n=4; Wet Igapo: n=3; Igarape: n=6; Transect: n=4).
831 Significant differences were tested using one-way analysis of variance with
832 Tukey's post hoc test at $P \leq 0.05$. Different letters above the bars indicate
833 significant differences between the treatments.
834 Fig. 6: NMDS analysis of (A) bacterial and (B) archaeal OTUs in soils from
835 different sites with different histories of inundation and from the Transect in
836 Cunia Reserve, determined (O) in the original soil, (I) after initial anaerobic
837 incubation, (D) after desiccation, and (R) after rewetting and second
838 incubation. The vectors indicate the contribution of OTUs to the ordination.
839 The number of each symbol and each color represents the number of true
840 replicates.
841 Fig. 7: The Ecological Station of Cuniã (ESEC) is located in Rondônia state,
842 Brazil, and has an official grid (25 x 25 km^2) used by research groups. The five
843 different soil conditions sampled were marked in the grid map. DF represents
844 the two Dry Forests; IG 1-7 represent the seven Igarapés sampled; IP 1-4
845 represent the four Wet Igapó forests, and IP 1, 2 and 4 are the Dry Igapó

forests; The transect samples were taken close to IG 3. The Grid Map was prepared using QGIS 2.18.