RESEARCH ARTICLE

Drying effects on archaeal community composition and methanogenesis in bromeliad tanks

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One sentence summary: Drying effects on microbial methane cycling in tank bromeliads of Guzmania species.

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ABSTRACT

Tank bromeliads are highly abundant epiphytes in neotropical forests and form a unique canopy wetland ecosystem which is involved in the global methane cycle. Although the tropical climate is characterized by high annual precipitation, the plants can face periods of restricted water. Thus, we hypothesized that water is an important controller of the archaeal community composition and the pathway of methane formation in tank bromeliads. Greenhouse experiments were established to investigate the resident and active archaeal community targeting the 16S rDNA and 16S rRNA in the tank slurry of bromeliads at three different moisture levels. Archaeal community composition and abundance were determined using terminal restriction fragment length polymorphism and quantitative PCR. Release of methane and its stable carbon isotopic signature were determined in a further incubation experiment under two moisture levels. The relative abundance of acetoclastic Methanosetaeaceae increased up to 34% and that of hydrogenotrophic Methanobacteriales decreased by more than half with decreasing moisture. Furthermore, at low moisture levels, methane production was up to 100-fold lower (≤0.1–1.1 nmol g dw⁻¹ d⁻¹) than under high moisture levels (10–15 nmol g dw⁻¹ d⁻¹). The rapid response of the archaeal community indicates that the pathway of methane formation in bromeliad tanks may indeed be strongly susceptible to periods of drought in neotropical forest canopies.

Key words: bromeliaceae; archaea; methanogenic pathway; drought

INTRODUCTION

Besides carbon dioxide (CO₂) and water vapor, methane (CH₄) is an important greenhouse gas with a global warming potential 25 times that of CO₂ (Denman et al., 2007). Sites of CH₄ formation are generally anoxic soils or sediments (Conrad, Klose and Claus 2002). Therefore, wetlands represent the largest source of CH₄ production by methanogenic archaea (Conrad 2009). However, known neotropical wetland sources do not sufficiently explain the observed amounts of emitted CH₄ over neotropical forest canopies (do Carmo et al., 2006; Frankenberger et al., 2008).

Tank bromeliads and other cryptic wetlands may contribute to the neotropical CH₄ budget (Martinson et al., 2010; Yavitt 2010). Tank bromeliads are common and highly abundant epiphytes in neotropical forest ecosystems. With their densely arranged leaves they create distinct habitats collecting wind-borne particles, leaf litter and rainwater for their nutrient demand (Zotz and Thomas 1999). The tanks are habitats for a diverse community of organisms, including microorganisms, insects and invertebrates (Laessle 1961; Carrias, Cussac and Corbara 2001; Greeney 2001) which support a complex food web for the
degradation of organic matter until the formation of CH$_4$ (Martinson et al., 2010). In many wetland ecosystems, CH$_4$ formation is due to CO$_2$-dependent and acetate-dependent methanogenesis. Nevertheless, the relative contribution of H$_2$/CO$_2$ and acetate to CH$_4$ production can vary considerably (Conrad 1999). The different pathways of methanogenesis result in different isotopic composition of the produced CH$_4$. The isotopic signatures can be used to constrain the global CH$_4$ budget and to determine the location and relative contribution of different CH$_4$ sources (Whiticar 1993). Although, archaea able for aceticlastic and hydrogenotrophic methanogenesis were detected in tank bromeliads (Martinson et al., 2010; Goffredi et al., 2011), the relative contribution of these two pathways to CH$_4$ production is not yet known.

Understanding the effects of biotic and abiotic factors influencing CH$_4$ formation in tank bromeliads. The unique morphology of tank bromeliads allows the plants to store water between their leaves which in turn creates anoxic niches for microbial CH$_4$ formation. Although the humid tropics are characterized by high annual rainfall, tank bromeliads can face periods of restricted water availability. Evaporation and rainfall periods of few hours may suffice to cause water stress for the plants and small individuals can completely dry out (Zotz and Hietz 2001). This occurs occasionally since the bromeliads lack an absorptive root system and thus are entirely dependent on precipitation for water supply (Smith et al., 1986). Thus, we hypothesized that water is an important controller of the archaeal community composition and CH$_4$ production in tank bromeliads. Furthermore, the effect of water availability may be increasing since increasing droughts are anticipated in the tropics as a result of anthropogenic climate change, and water deficiency in the tank bromeliads may intensify and become more frequent in this century (Salazar, Nobre and Oyama 2007; Cox et al., 2008; Malhi et al., 2008). Greenhouse experiments were established to investigate the resident and active archaeal community by targeting the 16S rRNA gene (16S rDNA) and the reversely transcribed 16S rRNA in composition and abundance in the tank slurry from a plant of the genus Guzmania. The incubations were conducted under different water amendments. Simulating a high or low moisture level, the tanks of plants were either watered every day (‘wet’) or incubated without any irrigation over one month (‘dry’). Additionally, archaeal community composition in the tank slurry was investigated under drying–rewetting stress by watering the tanks of the plants once per week (‘wet–dry’). In further experiments, CH$_4$ production and stable carbon isotopic signatures of CH$_4$ formed in the tank slurry were measured under two different moisture levels to determine the methanogenic pathways. Moisture revealed to be a major controller of archaeal community composition and CH$_4$ production from tank bromeliad slurry.

**MATERIAL AND METHODS**

**Greenhouse experiments**

The tank slurry is defined here as a mixture of organic matter and water accumulated in the various leaf axils of a single plant. For our experiments, we selected tank slurry from one individual plant of the species Guzmania squarrosa, sampled in Ecuador in the south of Loja [adjacent to Podocarpus National Park; for detailed site description see Martinson, Corre and Veldkamp (2013)] at 2000 m altitude in May 2011. Guzmania squarrosa is the most common bromeliad species at this site. The total tank slurry of the individual plant (348 g) was collected, pooled and immediately transported in cooled state (4°C) to the Max Planck Institute for Terrestrial Microbiology in Marburg, Germany, and stored at 4°C. Homogenized slurry (9 g fresh weight) was filled into each central tank of nine bromeliads of the species Aechmea fasciata ‘Primera’. Plants of A. fasciata ‘Primera’ are commercially available and have the advantage to possess one large central tank even at an early growth stage, thus enabling convenient and reproducible water amendment and sampling. Furthermore, plants of the genus Aechmea have frequently been used for greenhouse experiments to study hydrophysiology of the leaves and their acclimation to drought and recovery from dehydration (Londers et al., 2005; Ceusters et al., 2009). Tank bromeliads of A. fasciata ‘Primera’ were provided by Corn.Bak® B.V. (Asseldelf, Netherlands). The plants were 1 year old and of 10 cm size and had been cultivated with empty tanks in the greenhouse.

The experiment was performed in the greenhouse at a temperature of 25 ± 3°C. Different water amendments were conducted on three plants per treatment over 28 days. For treatment ‘wet’, plants were watered daily by spreading 2 ml deionized water in the central tank where the tank slurry was located. For treatment ‘wet–dry’, the plants were watered once per week with 2 ml deionized water. For treatment ‘dry’, the slurry in the plants was not watered and slowly dried over the period of 28 days.

After 28 days, vertical profiles of oxygen (O$_2$) concentrations were measured in the tank slurries of the treatments ‘wet’ and ‘wet–dry’ using an oxygen microelectrode (OX50, Unisense, Aarhus, Denmark). In all treatments, the pH was measured in the tank slurry. The water content was determined gravimetrically by drying 1 g aliquot of each tank slurry at 65°C for 72 h.

Subsequently, the tank slurry was sampled for molecular analysis. The leaves were carefully detached and the tank slurry was homogenized. Per plant, two aliquots of 0.3 g (fresh weight) tank slurry were taken with a sterile spatula and stored at −80°C till nucleic acid extraction. In total, six samples were analyzed per treatment.

**Molecular analyses**

Nucleic acids were extracted using a modified version of the protocol from Bürgmann et al., (2001). Briefly, the microbial cells in 0.3 g tank slurry were disrupted in a FastPrep®-24 (MP Biomedicals) beat-beating system. Total nucleic acids were subsequently recovered from the supernatant using a phenol/chloroform/isooamyl alcohol extraction (Sigma–Aldrich). The nucleic acids were precipitated with polyethylene glycol 6000 solution (Carl Roth) and re-dissolved in 100 μL of nuclelease-free water (Invitrogen). The sample was divided and half of the nucleic acids solution (50 μL) was stored at −80°C for later DNA-based molecular analyses. The remaining 50 μL were treated with RNase-free DNase (Qiagen) for removal of DNA in order to obtain pure RNA. The RNA was further purified using the RNasy Mini Kit (Qiagen). Finally, the RNA was precipitated with 96% ethanol (Carl Roth), resuspended in 20 μL of nuclelease-free water and stored at −80°C. The quality of the RNA was checked using a 1.5% w/v agarose gel (Biozym Scientific GmbH). The RNA
concentration was determined using a NanoDrop1000 instrument (Thermo Fisher Scientific). For reverse transcription of RNA into cDNA random hexamer primers (Roche) and SuperScript™ III reverse transcriptase (Invitrogen) were used.

All the following molecular approaches were conducted targeting the archaeal 16S rRNA gene (16S rDNA) and reversely transcribed archaeal 16S rRNA to investigate the resident and active archaeal community in tank slurry.

Analyses of terminal restriction fragment length polymorphism (TRFLP) were conducted targeting archaeal 16S rDNA and the 16S rRNA as described by Chin, Lukow and Conrad (1999) using the primer combination Ar109f (Grosskopf, Stubner and Liesack 1998)/Ar912rt-FAM (Lueders and Friedrich 2003). The reverse primer was labeled with the fluorescent dye 6-carboxyfluorescein (FAM). The purified amplicons were digested using Taq enzyme (restriction site: 5’…T*CGA…3’, 65°C, 3 h, Fermentas). The purification of the fragmented DNA was performed using SigmaSpin™ Post-Reaction Clean-Up Columns (Sigma–Aldrich) following manufactures’ manual. The size separation was conducted on an ABI PRISM 3130 capillary Genetic Analyzer (Applera Deutschland GmbH) using the software Genescan 4.0 (Applied Biosystems). The TRFLP data were obtained by comparison with an internal DNA standard. The resulting TRFLP profiles were standardized as described in Dunbar, Ticknor and Kuske (2001) using the peak area. For comparison, see also Martinson et al., (2010).

One clone library based on archaeal 16S rDNA and one based on reversely transcribed archaeal 16S rDNA sequences were generated from pooled triplicates of each treatment (‘wet’, ‘wet–dry’, ‘dry’) using the same primer combination for TRFLP without fluorescent dye. A total of 272 clones were sequenced by GATC Biotech AG (Germany) using the pGEM®-T Easy Vector System (Promega).

All sequences (272) were checked and aligned using ARB program and classified by adding them to the SILVA108 reference tree by parsimony (Pruesse et al., 2006). The identity of TRFs was assigned through performing in silico enzyme restrictions of clone library sequences. Since the same taxonomic groups were obtained from both approaches (16S rDNA and 16S rRNA), sequences of both libraries were listed in Table 1. All sequences that were generated in this study have been deposited in the GenBank nucleotide sequence database (accession numbers KM268122–KM268393).

The absolute numbers of archaeal 16S rDNA and reversely transcribed 16S rRNA copies were determined by quantitative PCR (qPCR) using the primer combination Ar364f (Burggraf, Huber and Stetter 1997) and Ar934br (Grosskopf, Stubner and Liesack 1998). The qPCR was set up in 96-well micro tier plates (BioRad). Each qPCR reaction contained in a total volume of 25 μL 1x SYBR® Green Ready Mix™ (Sigma), 3 mM MgCl₂ (Sigma), 0.66 μM of each primer and 1 μM FITC (fluorescein isothiocyanat, BioRad) as well as 2 μL of template. Negative controls without matrix DNA were run in parallel to ensure purity of the used reagents. The quantification standard was applied in a dilution series with 10⁻¹⁻¹⁰ gene copies. The standard was prepared from a clone containing the archaeal 16S rDNA as a plasmid insert. The following PCR program was used: 94 °C for 8 min followed by 45 cycles of 94 °C for 20 s, 50 °C for 20 s, 72 °C for 50 s for annealing, extension and signal reading. Afterwards, melting curves were performed to ensure purity of PCR products.

Dual-labeled probe-specific qPCRs were conducted to quantify the 16S rDNA of Methanosetaeaceae and Methanobacteriales (Yu et al., 2005). Each reaction was conducted in a volume of 25 μL containing the following mixture: 12.5 μL JumpStart Taq ReadyMix, 4 mM MgCl₂ 0.8 ng μL⁻¹ BSAl (Ambion), 0.5 μM of each primer, 0.2 μM dual-labeled probe and 5 μL template. The standards for this assay were prepared from a clone containing a 16S rDNA affiliated with Methanosetaeaceae or Methanobacteriales as a plasmid insert. The following PCR program was used: 94 °C for 10 min, followed by 45 cycles of 94 °C for 10 s and 60 °C for 30 s for annealing, extension and signal reading.

Table 1. Phylogenetic affiliation of distinct TRFs to archaeal 16S rDNA/rRNA clone sequences retrieved from tank bromeliad slurry of G. squarrosa

<table>
<thead>
<tr>
<th>Phylogenetic lineage</th>
<th>TRF (bp)</th>
<th>No. of clones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanobacteriales</td>
<td>92</td>
<td>20</td>
</tr>
<tr>
<td>Methanosarcinaceae</td>
<td>187</td>
<td>5</td>
</tr>
<tr>
<td>Methanocellaceae</td>
<td>259</td>
<td>1</td>
</tr>
<tr>
<td>Methanosaetaceae</td>
<td>284</td>
<td>149</td>
</tr>
<tr>
<td>Thermoplasmatales</td>
<td>382</td>
<td>18</td>
</tr>
<tr>
<td>Methanomicrobiales</td>
<td>393</td>
<td>11</td>
</tr>
<tr>
<td>Crenarchaeota</td>
<td>187</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>736</td>
<td>55</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>272</td>
</tr>
</tbody>
</table>

Laboratory incubations

Flask experiments were established for determining the methanogenic potential of tank slurry under different moisture contents. The ‘wet’ treatment had a water content of 28%. Simulating ‘dry’, the tank slurry from G. squarrosa (see above) was air dried for one week. The water content had then decreased from 28 to 15%. Each treatment was set up in triplicates with 9 g slurry filled into 125 mL glass flasks that were gas-tightly closed with screw caps including rubber stoppers. The flasks were flushed with synthetic air (21% O₂/79% N₂) for 5 min and then incubated in the dark at 25 °C for 21 days, until the concentration of CH₄ reached a plateau. Gas samples of 250 μL were taken 2–3 times per week from the headspace using a gas-tight pressure lock syringe (Vici, Baton Rouge, LA, USA) and analyzed immediately. Methane and CO₂ concentrations were analyzed using a gas chromatograph equipped with a methanizer (Ni-catalyst at 350 °C, Chrompack, Varian Deutschland GmbH, Darmstadt, Germany) and a flame ionization detector (Shimadzu Deutschland, Duisburg, Germany). Gas production rates were monitored during defined time periods as indicated in Fig. S1 (Supporting Information). Oxygen was supplemented 2–3 times per week maintaining atmospheric levels.

Ratios of carbon isotopes ⁰¹³C, ⁰¹₂C in CH₄ and CO₂ were measured using a gas chromatograph combustion isotope ratio mass spectrometer as previously described by Conrad, Claus and Casper (2009). The contribution of hydrogenotrophically formed CH₄ to total CH₄ production was calculated after Conrad (2005).

Briefly, the apparent fractionation factor (α_app) was calculated by

\[
\alpha_{app} = (\delta_{CO_2} + 1000)/(\delta_{CH_4} + 1000).
\]

\(\delta_{CO_2}\) and \(\delta_{CH_4}\) are the isotopic signatures of the carbon in the CO₂ and CH₄ respectively. Often α_app is expressed as enrichment...
factor $\epsilon_{\text{app}}$ (in %/unit) which can be determined through:

$$\epsilon_{\text{app}} = 1 - \alpha. \tag{2}$$

The relative contribution of the $\text{H}_2 + \text{CO}_2$-derived $\text{CH}_4$ ($f_{\text{H2}}$) was calculated with the following equation:

$$f_{\text{H2}} = (\delta_{\text{CH4}} - \delta_{\text{ma}})/(\delta_{\text{mc}} - \delta_{\text{ma}}). \tag{3}$$

where $\delta_{\text{ma}}$ was determined by

$$\delta_{\text{ma}} = \delta^{13}\text{C}_{\text{org}} + \epsilon_{\text{ac.CH4}} \tag{4}$$

and $\delta_{\text{mc}}$ by

$$\delta_{\text{mc}} = \delta_{\text{CO2}} + \epsilon_{\text{CO2.CH4}}. \tag{5}$$

$\delta_{\text{CH4}}$ was the $\delta^{13}\text{C}$ of $\text{CH}_4$ measured in the headspace. For calculation of $\delta_{\text{ma}}$, we assumed $\delta_{\text{acetate}}$ to be equal to $\delta^{13}\text{C}_{\text{org}}$ and $\delta_{\text{ac.methyl}} - \delta_{\text{acetate}} = -8\permil$ (Conrad et al., 2014a). Further, we assumed $\epsilon_{\text{ac.CH4}} = -10\permil$ (Penning et al., 2006). Average values for $\delta^{13}\text{C}_{\text{org}}$ in the slurry were $-28\permil$ (analyzed at the Centre for Stable Isotope Research and Analysis (KOSI) at the University of Göttingen, Germany, courtesy of Jens Dyckmans), thus resulting in $\delta_{\text{ma}} = -46\permil$. For $\delta_{\text{mc}}$, a value of $-85\permil$ that was typically obtained in incubation experiments, in which acetilactic methanogenesis was inhibited by 2% methyl fluoride (Conrad, Claus and Casper 2009).

Acetate concentration in tank bromeliad slurry was determined after 21 days of incubation. Prior to measurement, the tank content was mixed with distilled water at a ratio of 1:2 and shaken for 1.5 h at 25°C. The supernatant was sampled with a sterile syringe, membrane-filtered (0.2 μm) and stored frozen until analysis of acetate concentration using high-performance liquid chromatography (Sykam, Gilching, Germany) with refractive index and UV detectors (Krumböck and Conrad 1991). Acetate concentration in the supernatant was determined using dilution series of an external standard solution containing 1 mM acetate (Sigma–Aldrich).

A similar incubation experiment was set up using tank slurry from one adult individual plant of $G.\ gloriosa$ growing at 3000 m altitude. This bromeliad species is very common at this elevation. Treatment ‘dry’ had a moisture content of 16%, treatment ‘wet’ of 48%. In this experiment, 45 g of slurry was filled into 500 ml serum bottles ($n = 3$ per treatment) and incubated in the dark at 25°C for 15 days, until the concentration of $\text{CH}_4$ reached a plateau. Gas measurements, oxygen supplements, determination of isotopic signatures and methanogenic pathway were conducted as described above. Gas production rates were determined during defined time periods as indicated in Fig. S2 (Supporting Information).

**RESULTS**

Greenhouse experiment

Gravimetric water content of the tank slurry was at the end of the greenhouse incubation: 29 ± 1% in treatment ‘wet’, 6 ± 3% in treatment ‘wet–dry’ and 1 ± 0.5% in treatment ‘dry’. Microsensor measurements showed that in the ‘wet’ treatments $O_2$ concentrations decreased from atmospheric concentration at the surface of the tank slurry to undetectable concentrations at 2–3 mm depth and below. However, tank slurry of ‘wet–dry’ treatments still showed an atmospheric $O_2$ concentration at 3.5 mm depth. The pH values of the tank slurries were slightly higher in ‘wet’ treatments (pH 5.3) than in ‘wet–dry’ or ‘dry’ treatments (pH 4.9). Copy numbers of archaeal 16S rDNA and reversely transcribed 16S rRNA were significantly and oppositely affected by the different water amendments (Fig. 1). Archaeal 16S rDNA copy numbers were highest in the ‘wet’ treatment, while copy numbers of archaeal 16S rRNA were lowest in this treatment. A combination of TRFLP fingerprinting with cloning and sequencing was used to identify the archaeal populations under different moisture levels. TRFLP patterns (Fig. S3, Supporting Information) of the archaeal 16S rDNA and 16S rRNA in tank slurry were significantly affected by the different water amendments. The effect of moisture is highlighted in NMDS plots showing the distinct clustering of the resident (Fig. 2a) and active (Fig. 2b) archaeal community according to their treatment. Phylogenetic analysis of clone sequences revealed the presence of Methanobacteriales, Methanosarcinaeae, Methanococcales, Methanomicrobiales, Thermoplasmatales and Crenarchaeota in tank bromeliad slurry (Table 1). Methanocellulase were only detected as one clone sequence. The major terminal restriction

[Figure 1. Copy numbers of archaeal 16S rDNA and 16S rRNA in tank bromeliad slurry of $G.\ squarrosa$ under different moisture contents after 28 days. Error bars represent standard deviations (SDs) ($n = 3$). Different letters indicate significant differences between 16 rDNA (lower letters) and 16S rRNA copy numbers (capital letters) (ANOVA with Tukey’s post hoc test at $P < 0.05$).]
fragments (TRFs) were those with 92, 187, 284, 382, 393 and 736 bp lengths, which were found in all treatments and could be assigned to different archaeal lineages as shown in Table 1. Solely the 187 bp TRF was affiliated to more than one lineage (Table 1). Methanocellaceae were not detected by TRFLP analysis. The microbial community profiles of tank slurry showed that the relative abundance of major TRFs changed with moisture level. The relative abundances of single TRFs and affiliated lineages affected by moisture levels are shown in ternary plots (Fig. 3). Based on 16S rDNA data, the most prominent group was Crenarchaeota (736-bp TRF), which showed similar signal intensities in tank bromeliad slurry under all water amendments (Fig. 3a). In contrast, based on 16S rRNA data, the 736 bp TRF decreased in relative abundance and was mainly present in slurry of lower moisture (Fig. 3b). The same phenomenon was observed for the 187 bp TRF, which was affiliated to clones assigned as Crenarchaeota and Methanosarcinaceae. Further prominent TRFs were presented by the fragments of 92 and 284 bp lengths, assigned to the groups of Methanobacteriales and Methanosetaeae, respectively. These groups were significantly affected by moisture (Fig. 4). Based on 16S rDNA data, the group of Methanobacteriales (92-bp TRF) was dominating with 34 ± 3% relative abundance in the tank slurry of highest moisture. With decreasing water content, their relative abundance decreased by more than half (Fig. 4a). The same trend was observed using 16S rRNA data (Fig. 4b). In contrast, the abundance of Methanosetaeae (284 bp TRF) increased with decreasing moisture (Fig. 4c and d). Based on 16S rRNA data, the relative abundance of Methanosetaeae doubled from 9 ± 5% in treatment ‘wet’ to 23 ± 4% in treatment ‘wet–dry’ and tripled to 34 ± 4% in treatment ‘dry’ (Fig. 4d). The increasing dominance of Methanosetaeae with decreasing gravimetric water content was also seen as absolute abundance increased, as shown by using a probe-specific qPCR assay. The 16S rDNA copy numbers of Methanosetaeae doubled from 1.5 × 10^5 ± 2.5 × 10^4 copies gdw^{-1} in the ‘wet’ samples to 2.9 × 10^5 ± 5.3 × 10^4 copies gdw^{-1} in the ‘wet–dry’ and tripled to 4.8 × 10^5 ± 1.2 × 10^6 copies gdw^{-1} in the ‘dry’ samples. Contrary, the 16S rDNA copy numbers of Methanobacteriales were higher in the ‘wet’ samples (6.2 × 10^5 ± 1.1 × 10^5 copies gdw^{-1}) than in the ‘wet–dry’ (3.9 × 10^4 ± 1.2 × 10^4 copies gdw^{-1}) or ‘dry’ samples (1.2 × 10^5 ± 1.9 × 10^4 copies gdw^{-1}).

Laboratory experiments

The CH_4 production rate in tank slurry (sampled from G. squarrosa) was determined at two different water contents. After three weeks of incubation, the tank slurries with high moisture content showed higher acetate concentrations (64 ± 32 μM; n = 3) than those with low moisture contents (32 ± 6 μM; n = 3). Gas concentrations of CH_4, CO_2 and O_2 in the headspace of incubation flasks are shown in Fig. S1 (Supporting Information).
Methane production rates ranged between 10 and 15 nmol CH₄ g dw⁻¹ d⁻¹ and 0.05 and 1.1 nmol CH₄ g dw⁻¹ d⁻¹ in the tank slurries at high (28%) and low (15%) moisture content, respectively. The δ¹³C values of newly produced CH₄ were between −71 and −73‰ at high and between −50 and −66‰ at low moisture content. Rates of CO₂ production were higher at low (4.3–4.6 μmol g dw⁻¹ d⁻¹) than at high moisture (2.1–2.5 μmol g dw⁻¹ d⁻¹).

Concentrations of CH₄, CO₂ and O₂ in the headspace of incubation flasks with tank slurry from G. gloriosa were determined over 15 days (Fig. S2, Supporting Information). Methane production rates were 11 to 13 nmol CH₄ g dw⁻¹ d⁻¹ at high and ≤0.1 nmol CH₄ g dw⁻¹ d⁻¹ at low moisture content (Fig. 5b). The δ¹³C values for CH₄ ranged from −80 to −81‰ at 48% water content and from −56 to −63‰ at 16% water content. Release rates of CO₂ showed similar results as for G. squarrosa with 4.3–5.2 μmol g dw⁻¹ d⁻¹ at low moisture and 1.5–1.7 μmol g dw⁻¹ d⁻¹ at high moisture.

The δ¹³C values of CO₂ in the headspace were similar under all treatments and amounted to −28.5 and −29.5‰ to −29.6 and −29.7‰ for the tank slurries of G. squarrosa and G. gloriosa, respectively. The isotopic signatures of CO₂ and CH₄ were used to estimate the apparent enrichment factors ϵapp for the two different tank slurries at high and low moisture level (Table 2). The values were consistently lower under wet than under dry conditions indicating that the relative contribution of the methanogenic pathways was different (Table 2). Indeed, the relative fraction of hydrogenotrophically derived CH₄ was significantly higher in wet (fH₂ ≥ 0.68) than in dry tank slurry (fH₂ ≤ 0.35).

**DISCUSSION**

Moisture was identified to be a major determinant of the archaeal community composition in tank bromeliad slurry of Guzmania. For soil ecosystems, it has already been shown that...
moisture can be a dominant driver of bacterial community composition (Evans, Wallenstein and Burke 2014) and that it is important for microbial functioning and activity (Skopp, Jawson and Doran 1990; Schimmel et al., 1999; Evans and Wallenstein 2012). Especially for tank bromeliads, the storage of rainwater is a key parameter to ensure their supply over the ground. The tanks are inhabited by many aquatic and terrestrial organisms, and water volume in tank bromeliads has already been identified to shape the diversity of inhabiting insects (Armbruster, Hutchinson and Cotgreave 2002). Our results clearly show that the archaeal community composition and size in tank bromeliad slurry changed upon drying. Interestingly, archaeal communities in rice fields as man-made wetland ecosystems are often described as relatively resistant against dramatic changes like drainage or crop rotation (Lueders and Friedrich 2000; Krüger et al., 2005; Watanabe, Kimura and Asakawa 2006; Ma, Conrad and Lu 2012; Fernandez Scavino et al., 2013). Stress resistance of microbial communities can be influenced by various key factors including microbial richness and evenness (Wittebolle et al., 2009) as well as the historical soil moisture regime which may affect the magnitude and timing of microbial community response to drying and rewetting events (Evans and Wallenstein 2012). Accordingly, an increased resistance to drought was observed for microbial communities in grassland (de Vries et al., 2012) when the soil was previously exposed to drying–rewetting events and a poor adaption to drying–rewetting events when the soil lacked a history of drought (Thion and Prosser 2014). Although tank bromeliads and Guzmania spp. in general are very efficient in maintaining moisture in their tanks (Zotz and Thomas 1999), the plants can be exposed to periods of droughts. However, the levels of relative air humidity at our study sites at 2000 and 3000 m elevation remain fairly high over the whole year with daily minima of 15 to 30% (Moser, Hertel and Leuschner 2007) and dry conditions become only significant for short periods during intensive summers (Peters et al., 2013). Therefore, our drying experiments presently do not represent normal in situ conditions but the potential under extreme conditions. Likewise, drying of Amazonian lake sediments resulted in changes of archaeal community composition (Conrad et al., 2014b). However, the high organic matter content in tank slurry (38–45%) may enhance the effect of drying on the archaeal tank community. Orwin and Wardle (2005) reported that soils rich in organic matter showed a reduced microbial resistance to drying. Soils with high C content have higher water retention and therefore microbial communities may be more protected from changes in moisture contents and less well adapted to drying (Rawls et al., 2003).

Forest humidity is decreasing since 1998 (Wilcke et al., 2013) and rainless periods of two or more weeks become more frequently at our study site (Peters et al., 2013). These changes are in line with climate change effects that are projected to lead to larger periods of drought in the neotropics (Hulme and Viner 1998; Oliveira et al., 2005). These drought-stressed periods in turn may affect rates of organic matter mineralization and production of CH₄ and CO₂ from bromeliad tanks. Here, CO₂ release rates into the headspace of incubation flasks were 2–3 times higher than from tank slurry of dry than of high moisture, indicating aerobic degradation of organic matter which is postulated to be faster than anaerobic degradation (Reddy and Patrick 1975). Methane production rates were up to 100 times lower in tank slurry with relatively low moisture content. Additionally, the methanogenic pathway and the archaeal community composition were affected by drought. Our results showed that extended periods of drought resulted in a decrease of the relative abundance of hydrogenotrophic Methanobacteriales in tank bromeliad slurry, and in an increase of the acetoclastic Methanosetaeaceae (Fig. 4). Consistently, analyses of stable carbon isotopic signatures indicated a decrease of hydrogenotrophic methanogenesis and an increase of acetoclastic methanogenesis with decreasing moisture of the tank slurry.

Our experiments showed that CH₄ was mainly produced by hydrogenotrophic methanogens in tank slurry at high moisture content. Relatively high contribution of hydrogenotrophic methanogenesis is characteristic for lake sediments (Crill and Martens 1983; Galchenko 1994; Namsaraev et al., 1995; Conrad et al., 2014b) and for the methanogenic degradation of aged organic carbon (Nakagawa et al., 2002). Furthermore, hydrogenotrophic methanogenesis has been found in environments in which acetate is assimilated rather than dissimilated such as in animal gut systems (Lange, Westermann and Ahring 2005; Liu and Whitman 2008) or microbial mats (Sandbeck and Ward 1981).

Our experiments further showed that the contribution of acetoclastic methanogenesis became more important, when the tank slurry became dry. Acetate concentrations at the end of the incubation experiment were also lower in the dry tank slurry. A slight increase of acetoclastic methanogenesis was also observed by Conrad et al., (2014b) in lake sediments and by Knorr, Oosterwoud and Blodau (2008) in a fen soil upon experimental drought. However, lower water tables or drought in our experiment simultaneously implied an increased oxygen exposure to microbial organisms. Methanogenic archaea are described as strict anaerobes (Zinder 1993) and their metabolism involves many O₂-sensitive redox centers (Zehnder 1978). Nevertheless, it has also been shown that aerated soils are inhabited by active methanogens (Peters and Conrad 1995; Angel, Mattheis and Conrad 2011), mainly members of the Methanocellaceae and Methanosarcinaceae (Angel, Mattheis and Conrad 2011). Subsidiary, Methanosarcinaceae was solely detected in tank slurry of low moisture based on 16S rRNA analysis (Fig. 3b). For Methanocellaceae and Methanosarcinaceae, it is known that they possess a large diversity of genes encoding for oxygen detoxifying enzymes (Erkel et al., 2006; Angel, Mattheis and Conrad 2011). Some of these enzymes (catalase, superoxide dismutase, rubrythrin, peroxiredoxin) were also found in the genomes of Methanosaeta concilii (NCBI reference sequence: NC_015416.1; Barker et al., 2011) and in M. harundinacea (NCBI reference sequence: CP003117; Zhu et al., 2012). We therefore assume that the increase of Methanosarcinaceae in the dry and therefore aerated tank slurry indicates that Methanosarcinaceae are also more tolerant to O₂ than previously assumed.

Beside methanogenic archaea, Crenarchaeota were dominantly present in tank bromeliad slurry based on 16S rDNA
analysis. However, targeting the 16S rRNA and so the more active members of the archaean community Crenarchaeota play a minor role in contrast to the methanogenic community (e.g. Methanobacteriales, Methanosaetaeae). Members of Crenarchaeota have previously been detected in relatively high numbers in different soils (Timonen and Bomberg 2009). Nevertheless, we do not know which function Crenarchaeota may have there and particularly in tank slurry.

In conclusion, our study provides evidence that moisture in the tank slurry is a dominant driver of the archaean community composition, the archaean abundance as well as the methanogenic pathway. With increasing drought, the methanogenic community shifted from a hydrogenotrophic-dominated community, represented by Methanobacteriales, to an aceticlastic-dominated community, represented by Methanosae-taceae. This shift was accompanied by an increase of aceticlastically produced CH₄. Hence, we suggest that microbial methane cycling in bromeliad wetlands is strongly susceptible to periods of drought in neotropical forest canopies. In order to confirm our assumption, it will be necessary to investigate microbial CH₄ cycling in bromeliad tanks of other bromeliad species and at different sites with different moisture regimes.

SUPPLEMENTARY DATA
Supplementary data is available at FEMSEC online.

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