Community structure – ecosystem function relationships in the Congo Basin methane cycle depend on the physiological scale of function

Kyle M. Meyer[1#*+], Anya M. Hopple[1#], Ann M. Klein[1], Andrew H. Morris[1], Scott Bridgham[1], Brendan J. M. Bohannan[1]

1. Institute of Ecology and Evolution, University of Oregon, Eugene, OR, USA

# Authors contributed equally to the preparation of this manuscript

*Corresponding author: kmmeyer@berkeley.edu

+ Present address: Department of Integrative Biology, University of California – Berkeley, Berkeley, CA, USA

Keywords: Biodiversity-Ecosystem Function, Congo Basin, Methane, Microbial ecology

Running title: Community structure – function relationships

ABSTRACT

Belowground ecosystem processes can be highly variable and difficult to predict using microbial community data. Here we argue that this stems from at least three issues: 1) complex covariance structure of samples (with environmental conditions or spatial proximity) can make distinguishing biotic drivers a challenge, 2) communities can control ecosystem processes through multiple mechanisms, making the identification of these controls a challenge and 3) ecosystem function assessments can be broad in physiological scale, encapsulating multiple processes with unique microbially mediated controls. We test these assertions using methane (CH_4)-cycling processes in soil samples collected along a wetland-to-upland habitat gradient in the Congo Basin. We perform our measurements of function under controlled laboratory conditions and include environmental covariates in statistical analyses to aid in identifying biotic drivers. We divide measurements of microbial communities into four attributes (abundance, activity, composition, and diversity) that represent different forms of community control. Lastly, our process measurements differ in physiological scale, including broader processes (gross methanogenesis and methanotrophy) that involve more mediating groups, to finer processes (hydrogenotrophic methanogenesis and high-affinity CH_4 oxidation) with fewer mediating groups. We observed that finer scale processes can be more readily predicted from microbial community structure than broader scale processes. In addition, the nature of those relationships differed, with broad processes limited by abundance while fine-scale processes were associated with diversity and composition. These findings demonstrate the importance of carefully defining the

47 physiological scale of ecosystem function and performing community
48 measurements that represent the range of possible controls on ecosystem
49 processes.
50
51
52
53
54
55
56
57
58
59
60
61
62
63
64
65
66
67
68
69

INTRODUCTION

Belowground ecosystem processes can be highly variable, and there have been numerous attempts to explain or predict this variation using microbial community data. However, our ability to identify microbial community drivers of processes remains in its infancy. This challenge stems from at least three issues. First, community variables often have complex covariance structure (e.g. spatial or environmental autocorrelation) (Legendre, 1993), which can complicate the task of distinguishing the biotic signal underlying ecosystem process rate variation. Second, communities can control processes through a number of mechanisms, making the identification of these controls a challenge (Bier et al., 2015). For example, communities can influence the rate of a process through abundance (of particular groups or the community as a whole), composition (i.e. the identity of community members), diversity, and activity, each of which would require the quantification of different community attributes. Lastly, function is often assessed at a broad physiological scale that encapsulates many processes that could have unique controls (Inkpen et al., 2017). For example, gross decomposition of organic matter or net flux of methane (CH_4) into the atmosphere are commonly measured belowground ecosystem functions, yet these measurements represent the aggregate result of many different physiological processes.

We tested this idea using the two counteracting microbial processes that largely control atmospheric CH_4 emissions from soils: CH_4 production (methanogenesis) and CH_4 consumption (methanotrophy). Each of these processes can be further divided into underlying processes that involve fewer mediating

93 microbial taxa. For instance, gross methanogenesis can have direct inputs from at
94 least three processes: hydrogenotrophic (which uses CO_2 and H_2 to produce CH_4),
95 acetoclastic (which uses acetate), and/or methylotrophic (which uses methanol,
96 methyl-amides, or methyl-sulfides) methanogenesis. The process of methanotrophy
97 can be subdivided as well. Methanotrophy at high CH_4 concentrations (typical of
98 those found in waterlogged soil and sediment) is performed by a wide diversity of
99 methanotrophs found in the Gammaproteobacteria, Alphaproteobacteria, and
100 Verrucomicrobia (Knief, 2015), whereas the consumption of CH_4 at atmospheric
101 concentrations (a.k.a. high-affinity CH_4 oxidation) appears to be much more
102 phylogenetically restricted and is likely performed by many fewer taxa (Ho et al.,
103 2013; Knief, 2015; Kolb, 2009). Taken together, these observations suggest that
104 gross measurements of either methanogenesis or methanotrophy may mask subtle
105 variations in these underlying processes and could thereby blur the connection
106 between community variation and ecosystem function.

107 A major challenge in the study of ecosystem function lies in identifying
108 underlying community controls. Group abundance is one such form of control,
109 whereby the rate of a process only proceeds as quickly as the total number of
110 capable individuals. There are examples of this control for both methanogenesis
111 (Ma, Conrad, & Lu, 2012) and methanotrophy (Freitag & Prosser, 2009), but many
112 studies have found no such relationship (see Rocca et al., 2014). While this could be
113 driven in part by technical issues (e.g. abundance being inaccurately assessed due to
114 primer bias), processes may be controlled by other community attributes. Most soil
115 organisms are in an inactive state at any given time (Lennon and Jones, 2011). Low

116 activity levels can limit the rate of ecosystem processes either by decreasing
117 syntrophic interactions or preventing substrate uptake. Measurements of
118 methanotroph and methanogen transcriptional activity levels have been shown to
119 positively correlate with process rates (Freitag & Prosser, 2009; Freitag, Toet,
120 Ineson, & Prosser, 2010), suggesting that the activity levels of key groups can
121 constrain these processes. Moreover, community assessments using RNA may more
122 closely approximate the active fraction of the community (Jones & Lennon, 2010;
123 Kamke et al., 2010; Baldrian et al., 2012; although see Papp et al., 2018a) and
124 therefore provide a means to approximate activity-based community controls.
125 The composition of a community can impose another constraint on
126 ecosystem processes. Composition control (a.k.a. species identity effects (Bannar‐
127 Martin et al., 2018)) could occur if certain community members perform a process
128 more efficiently or rapidly than others, and those taxa/traits are dispersal limited.
129 In this case, certain individuals would be expected to more strongly correlate with
130 process rate variation than others. Distinguishing such a compositional signal can be
131 a challenge because communities tend to co-vary depending on their spatial
132 proximity or similarity in environmental controls (Legendre, 1993). There is
133 precedent to suggest that community composition is an important determinant of
134 CH_4-cycling rates. For instance, even closely related methanogen/methanotroph
135 taxa can differ in traits such as substrate affinity, substrate preference,
136 environmental tolerance, or competitive ability (Garcia, Patel, & Ollivier, 2000; Ho et
137 al., 2013; Knief, 2015; Nazaries, Murrell, Millard, Baggs, & Singh, 2013). Therefore,
138 differences in community composition have the potential to influence CH_4-cycling

139 processes, which has been shown in several systems (Bodelier et al., 2013; McCalley
140 et al., 2014; Nazaries, Pan, et al., 2013; Sierocinski et al., 2017, 2018). Membership of
141 other functional guilds within a community is also an important consideration,
142 particularly for processes that require metabolic byproducts as the primary
143 substrate. Methanogens depend on the fermentative byproducts (*e.g*. H^+ and
144 acetate) of other community members and must compete for these substrates with
145 other community members. This is also the case for methanotrophs, where
146 competition for O_2 and/or other soil nutrients can limit rates of methanotrophy
147 (Bodelier, Roslev, Henckel, & Frenzel, 2000). Thus, *who is there* may be a
148 particularly important consideration for predicting the rates of ecosystem
149 processes, particularly if efficient traits are dispersal limited or competition for
150 substrates is common.

151 Lastly, a growing body of work suggests that biodiversity may affect rates of
152 ecosystem processes (Tilman, Wedin, & Knops, 1996), as well as the resistance,
153 resilience, or stability of a process through time (Loreau et al., 2001; Tilman &
154 Downing, 1994). This pattern could arise through multiple ecological mechanisms
155 including: 1) niche complementarity, 2) sampling effects, or 3) facilitation. It has
156 been argued that ecosystem functions mediated by a wide assortment of taxa may
157 be less influenced by levels of diversity than those mediated by relatively fewer taxa
158 (Schimel, 1995; Schimel & Gulledge, 1998). This would suggest that as one narrows
159 the physiological scale of ecosystem function, the diversity of participants should
160 become a stronger determinant of process rates. The abilities to produce and
161 consume CH_4 are both highly phylogenetically conserved (Martiny, Treseder, &

Pusch, 2013), and relatively few taxa possess the ability to carry out these processes. There are even fewer taxa that perform underlying processes such as hydrogenotrophic methanogenesis and high-affinity methanotrophy (Knief, 2015; Kolb, 2009). Positive diversity – ecosystem function relationships have been observed for methanotrophy experimentally using intact soil cores from the field (Bodelier et al., 2013), as well as in artificially assembled methanotroph communities (Schnyder, Bodelier, Hartmann, Henneberger, & Niklaus, 2018). This has also been observed for methanogenesis in anaerobic digesters (Sierocinski et al., 2018). Although several studies have tested whether diversity and process rates are related, few have asked whether adjusting the physiological scale at which function is assessed improves this relationship.

Here we present results from a set of experiments designed to identify the biological drivers of variation in CH_4-cycling dynamics. Our goals were to determine (1) whether we could identify significant community controls on CH_4 cycling after accounting for covariance structure, and (2) whether the strength and nature of those controls changes with the physiological scale of the process. To do this, we collected soils along a diverse wetland-to-upland habitat gradient in the Congo Basin, a region that has received very little scientific attention for its role in the global CH_4 cycle (Bridgham, Cadillo-Quiroz, Keller, & Zhuang, 2013; Kirschke et al., 2013) and has been shown to exhibit highly dynamic spatial and temporal CH_4 cycling dynamics (R. A. Delmas, Tathy, & Cros, 1992; R. Delmas, Servant, Tathy, Cros, & Labat, 1992; MacDonald et al., 1999; Tathy et al., 1992). We estimate process rate potentials under controlled laboratory conditions, which minimizes the impact of

environmental variation and enhances the biotic signal underlying process rate variation. We varied the physiological scale at which we assessed ecosystem function, ranging from broader scales including gross methanogenesis and methanotrophy, to finer scales including hydrogenotrophic methanogenesis and high-affinity methanotrophy. We paired RNA- and DNA-based microbial community measurements with each process measurement and calculated four sets of community attributes (abundance, activity, composition, and diversity) to aid in the identification of putative community controls. We hypothesized that there would be a connection between microbial community structure and CH_4-cycling processes after accounting for environmental variation, and that finer scale processes (*i.e.* those involving a lower diversity of mediating taxa) would exhibit a stronger relationship with community attributes than broader processes, as exemplified by a higher proportion of variance explained. Our results suggest that communities are an important driver of CH_4-cycling processes in tropical ecosystems, and that the strength and nature of the relationship between community structure and ecosystem function can depend on the physiological scale by which function is assessed.

METHODS AND MATERIALS

Site selection and sampling

We selected a diverse set of 15 sites in Southwestern Gabon in and around the Gamba Complex of Protected Areas (Lee, Alonso, Dallmeier, Campbell, & Pauwels, 2006). Gabonese ecosystems belong to the Guineo-Congolian regional

center of endemism (White, 1979), making them broadly representative of the Congo Basin. Our sites included 4 mineral-soil forested wetlands, 2 peatlands, 2 seasonally wet mineral-soil forests, 3 upland grasslands (2 with termite mounds), 2 upland forests, a 1-year old plantation, and 1 abandoned plantation. Seasonal wetlands were identified by tree species composition, thick O and/or A soil horizons with mottling, and tree watermarks. Water tables were at least 40 cm below the soil surface in seasonal wetlands. Our sampling took place at the beginning of the Gabonese rainy season, from October to November 2014.

Potential gross and high-affinity CH_4 oxidation rates

We collected three intact soil cores from above the water table at the beginning, middle, and end of 35 m field transects established at each site. The samples were stored in PVC tubes (5 cm dia., 8 cm height) to maintain soil structure and kept at *in situ* air temperatures (28 °C) until the end of the sampling trip (~3 weeks). One week after returning to the University of Oregon, each PVC core was placed into a gas-tight Mason jar that was retro-fitted with a headspace sampling port and incubated at 28 °C in the dark. Rates of aerobic CH_4 oxidation were determined under initially low (5 ppm CH_4/mL in headspace) and high (1000 ppm CH_4/mL headspace) CH_4 concentrations, to estimate rates of high-affinity and gross methanotrophy, respectively. CH_4 concentration measurements were taken at 0.33, 3, 6, and 9 hours for high-affinity methanotrophy and at 0.33, 3, 6, 9, 24, and 48 hours for gross methanotrophy. The same soil cores were used to determine high-affinity methane oxidation and gross methanotrophy in quick succession. We

applied a first-order exponential decay function to determine the rate constant (k, units = d^{-1}; *i.e.* $dCH_4/dt = k[CH_4]$) of the exponential decrease in CH_4 when at low initial CH_4 concentrations (*i.e.* high-affinity methanotrophy). At high CH_4 concentrations we measured the linear decreases in CH_4 to determine the maximum velocity rates (V_{max}, units = μg CH_4 cm^{-3} d^{-1}) (*i.e.* gross methanotrophy). During our high-affinity CH_4 oxidation incubations, CH_4 concentrations exponentially decreased in all samples, except for one, across our four measurements; thus, all time points were included in subsequent calculations. While one sample did not immediately exhibit CH_4 oxidation at the first timepoint (0.33 hours), it did show exponential decreases in CH_4 concentrations following the 3 hour timepoint; thus, only three timepoints were used to calculate the k-value for this sample. During our gross methanotrophy incubations, we included only timepoints that encompassed the linear portion of the exponential decrease in CH_4 concentration, which ranged from 3-5 timepoints depending on the sample.

Potential gross methanogenesis and hydrogenotrophic pathway predominance

Across all wetland sites, we collected three intact soil cores in PVC tubes from 0 – 10 cm below the water table at the beginning, middle, and end of 35 m transects at each site. The samples were topped with site water and tightly sealed to ensure an anaerobic environment and transported to the University of Oregon. Anaerobic incubations took place at field temperatures (28 °C) within two days following arrival at University of Oregon. In a glove box filled with a N_2 atmosphere (<5% H_2 in the presence of palladium catalyst; Coy Laboratory Products, Grass Lake,

Michigan, USA), approximately 10 g of wet weight soil were added to 120 mL serum bottles and mixed with 10 mL of deoxygenated, deionized water. Sample bottles were then flushed with N_2 for 15 minutes to begin the incubation. Headspace samples were analyzed over the course of three days (0, 1, 2, and 3 days). Total CH_4 was calculated using Henry's Law, adjusting for solubility, temperature, and pH (Bridgham & Ye, 2013). CH_4 production rates were calculated using the linear accumulation ($r^2 > 0.90$ in all cases) of gas through time. To measure hydrogenotrophic methanogenesis, we used a $^{14}CO_2$ tracer method (Keller & Bridgham, 2007), which allowed us to distinguish the percent of total CH_4 that had been produced using CO_2/H_2 as the substrate (i.e. hydrogenotrophic methanogensis). This was done with a gas chromatograph fitted with a radioactive gas proportional counter (LabLogic Systems) simultaneously with the gross methanogenesis measurements over three days.

Soil physical and chemical analysis

We measured a suite of abiotic variables from each soil sample to incorporate into our community model as a covariate (see below). For the methanogenesis study, we recorded pH, total % nitrogen (N), organic carbon (C), moisture content, and bulk density. For the methanotrophy study, we measured N, C, moisture content, and bulk density. Soil pH was determined from a 1:1 soil to deionized water solution. Total % N and organic C were measured on a Costech ECS 4010 Elemental Analyzer (Valencia, CA), with each sample analyzed in duplicate. Moisture content (gravimetric water content) and bulk density were measured by

277 the change in weight of a soil sub-sample following 48 hours of drying at 60 °C.
278 Additionally, material from the three soil cores at each site was combined and
279 homogenized for texture analysis using the hydrometer method (Gavlak, Horneck,
280 Miller, & Kotuby-Amarcher, 2003), with 5% sodium hexametaphosphate as the
281 dispersing solution. Because soil texture measurements required pooling samples
282 from each site- and all other variables were measured on a per sample basis, texture
283 was not included in the environmental covariate calculation.
284
285 ***Soil RNA/DNA co-extraction and sequencing***
286 Soil DNA and RNA were co-extracted from Lifeguard-preserved soil samples
287 using MoBio's Powersoil RNA Isolation kit with the DNA Elution Accessory Kit
288 (MoBio, California, USA) following manufacturer's instructions. RNA was reverse
289 transcribed to cDNA using Superscript III first-strand reverse transcriptase and
290 random hexamer primers (Life Technologies, USA). Extractions were quantified
291 using Qubit (Life Technologies, USA). We amplified the V4 region of the 16S SSU
292 rRNA gene in sample DNA and cDNA using the primers 515F and 806R (Caporaso et
293 al., 2011). Sequencing libraries were prepped using a dual-indexing approach
294 (Fadrosh et al., 2014; Kozich, Westcott, Baxter, Highlander, & Schloss, 2013). In
295 short, each PCR reaction was performed using 12.5 µl NEBNext Q5 Hot Start HiFi
296 PCR master mix (New England Biolabs, USA), 11.5 µl gene-specific primer mix (1.09
297 µM of each primer), and 1 µl template (DNA or cDNA). A subset of samples was used
298 to find 1) the optimal primer annealing temperature, and 2) the minimum number
299 of cycles for adequate target amplification. For the 16S rRNA gene target this was

61° C and 20 cycles. The final reaction conditions were: 98° C 30 seconds (initialization), 98° C 10 seconds (denaturation), gene-specific annealing step for 20 seconds (see above), and 72° C for 20 seconds (final extension). Reactions were followed by magnetic bead purification using 20 µl Mag-Bind RxnPure Plus isolation beads (Omega Bio-Tek, USA). Reactions were quantified using Qubit (Life Technologies, USA) then were multiplexed at equimolar concentration. Final pooled amplicon libraries were sequenced using the Illumina MiSeq (300 paired-end) platform at the Oregon State University Center for Genome Research and Biocomputing facility.

Bioinformatic processing

Demultiplexed reads were joined using PEAR (version 0.9.10) with default parameters (Zhang, Kobert, Flouri, & Stamatakis, 2014) and quality filtered using Prinseq (version 0.20.4) (Schmieder & Edwards, 2011). Sequences with a mean quality score ≥ 30 and length 250-350 bp were retained. Sequences were dereplicated, denoised, and checked for chimeras using the DADA2 pipeline (Version 1.6) (Callahan et al., 2016) implemented in QIIME2 (Caporaso et al., 2010) (https://qiime2.org). For the methanogenesis study 17 of the 18 DNA-based samples, and 13 of the 18 RNA-based samples had sufficient quality to be included. For the methanotrophy study 41 of the 44 DNA-based samples, and 34 of the 44 RNA-based samples passed our quality filter. Taxonomy was assigned to the resulting amplicon sequence variants (ASVs) using the Ribosomal Database Project

online classifier (Wang, Garrity, Tiedje, & Cole, 2007) with database release 11.5 (Cole et al., 2014).

Quantitative PCR

We quantified the abundance of methanogens in each sample from which CH_4 production and hydrogenotrophic pathway predominance were measured. To do so we performed qPCR of the *mcrA* gene on sample DNA using the mlas-mcrA-rev primer combination (Steinberg & Regan, 2008). Samples were run on an ABI StepOnePlus thermocycler (ABI, USA), using Kapa SYBR reagents (Kapa Biosystems, USA) according to manufacturer recommendations. For each sample, 8 ng DNA was used, and the following amplification conditions were applied following optimization: 98° C 10 minutes, 98° C 15 seconds, 55.6° 15 seconds, 72° 60 seconds. A melt curve analysis was performed to verify target amplification. Not all samples reliably amplified; in the end we obtained useable *mcrA* quantifications for 12 of the 18 samples. Although attempted, the transcriptional activity of *mcrA* from soil cDNA did not produce reliable results, and thus is not included in this study. We used a similar approach to quantify methanotroph abundance and transcriptional activity by targeting the *pmoA* gene in the sample DNA and cDNA, respectively, using the A189 – mb661 primer combination (Bourne, Donald, & Murrell, 2001). Reactions were performed on a Bio-Rad CFX96 real-time qPCR instrument, using SsoAdvanced Universal SYBR Green supermix (Bio-Rad, USA) following manufacturer instructions. For each sample, 2 ng template were used with the following reaction conditions: 98° C 10 minutes, 98° C 15 seconds, 55.6° 15 seconds, 72° 60 seconds.

All samples were amplified in triplicate. In both cases, sample amplification was compared to a standard positive control to quantify total gene (or transcript) copy number. We obtained useable *pmoA* quantifications for 42 of the 44 DNA samples and 32 of the 44 RNA samples. In the case of *mcrA*, the positive control was an *mcrA* plasmid and, in the case of *pmoA*, we used purified DNA from strain *Methylococcus capsulatus* Foster and Davis (ATCC 33009D-5). We used LinRegPCR (Ramakers, Ruijter, Deprez, & Moorman, 2003; Ruijter et al., 2009) to process amplification data which allows for the calculation of individual PCR efficiencies. Individual PCR efficiencies did not significantly differ between habitat types, so gene copy was calculated using the average PCR efficiency of all reactions. Finally, gene copy (or transcript) numbers were normalized to the total ng DNA (or cDNA) used in the reaction.

Microbial community attribute calculations, statistical analysis, and comprehensive modeling

All statistical analyses were performed using R (version 3.4.4) (R Core Team, 2018). Community matrices for the methanotrophy experiments were rarefied ten times at 28,000 counts per sample, then averaged, and community matrices for the methanogenesis experiments were rarefied to 30,400 counts per sample ten times and averaged, in order to account for differences in sampling extent across samples. Additionally, these rarefied community matrices were subsetted for known methanogens/methanotrophs (Supplementary Table 1) to create functional group community matrices. We categorized community data into the following four

368 attributes (Table 1): 1) *Abundance* of methanogens/methanotrophs using qPCR of
369 *mcrA* and *pmoA* genes (described above) respectively, as well as the relative
370 abundance of known methanogens/methanotrophs in the DNA-inferred 16S-based
371 prokaryotic community, 2) *Activity* of methanogens/methanotrophs using the
372 relative abundance of methanogens and methanotrophs in the RNA-inferred
373 prokaryotic community, and qPCR of *pmoA* using soil cDNA (described above), 3)
374 *Composition* of the broader prokaryotic community or of subsets of known
375 methanogen/methanotroph taxa from the 16S-inferred community matrix
376 (described below), and 4) *Diversity* of the prokaryotic community as well as
377 methanogen/methanotroph community subsets using ASV-level richness and
378 Shannon diversity. Species richness and Shannon diversity were calculated using
379 the 'vegan' package (Oksanen et al., 2015) in R. Regression plots were created using
380 the package 'ggplot2' (Wickham, 2009), and 95% confidence interval bands were
381 plotted around each linear fit using the 'geom_smooth' function (option = 'lm').
382 Difference in process rates among wetlands and uplands was assessed using a
383 Kruskal-Wallis test.
384
385 *Associations between community attributes and CH_4-cycling processes*
386 Because communities tend to have complex covariance structure, we took
387 several measures to avoid spurious associations when distinguishing biotic
388 correlates of CH_4-cycling processes. First, our process measurements were
389 performed under constant temperatures similar to those experienced in the field
390 (described above). By doing so, we minimized the influence of certain

391 environmental factors (*e.g.* temperature fluctuations or precipitation events) on our
392 process rate measurements. Secondly, we added two covariates to our model when
393 assessing associations with process rate measurements: 1) a soil physicochemical
394 covariate, and 2) and a sample (community) covariate. The soil physicochemical
395 covariate was calculated using principle components analysis (PCA) on soil
396 physicochemical measurements (described above). This was done using the prcomp
397 function in the 'stats' package of R (R Core Team, 2018), with variables scaled to
398 unit variance to account for differences in units of measurement. Sample scores for
399 the first principle component (PC1) were used as the environmental covariate. We
400 applied a similar approach to calculate the community covariate. In this case, the
401 rarefied community matrix was Hellinger transformed, then ordinated using the
402 prcomp function while scaling to unit variance, in order to calculate the community-
403 based sample PC1 scores. By including these covariates in our model we are
404 regressing each community attribute against the residual variation of the CH_4-
405 cycling process of interest after accounting for the variation explained by
406 environment and underlying sample structure.
407 To assess associations between community membership and process rate
408 variation we used the regression approach described above on the relative
409 abundance of individual taxa (ASVs). Since performing this type of analysis on
410 thousands of individual taxa increases the risk of false positives, we adjusted our
411 significance threshold (alpha = 0.05) using a Bonferroni correction, i.e. by dividing
412 by the number of of taxa being tested. In cases where significant taxa were
413 identified, those taxa were subsetted from the rarefied community matrix, then

ordinated (following the above procedure) to reduce them to a single variable. The sample scores of the first principle component were subsequently used for regression against CH_4-cycling process rates. Linear regressions were performed using the lm function in the 'stats' package in R. Gross methanotrophy and methanogenesis rate data were log_2-transformed in order to satisfy assumptions of normality. For each relationship we report the adjusted R^2 and *p* value.

RESULTS

Identifying biotic drivers of CH_4-cycling processes

Methanogenesis processes

Gross Methanogenesis

Gross methanogenesis rates varied from 0.06 to 6.7 μmol CH_4 g $soil^{-1}$ d^{-1} (average 1.1 ± 1.5 μmol CH_4 g $soil^{-1}$ d^{-1}, Fig. 1A), with rates in mineral soil wetlands on average 3.8 times higher than peatlands. For breakdown of soil physico-chemical variables by habitat see Supplementary Table 2. We identified two community attributes that were associated with gross methanogenesis rate after accounting for sample and environmental covariance structure: methanogen abundance (*i.e.* the abundance of *mcrA* genes, Adj. R^2 = 0.47, *p* = 0.008, Fig. 1B, Table 2), and the alpha diversity of methanogens (Richness: Adj. R^2 = 0.25, *p* = 0.023, Shannon: Adj. R^2 = 0.23, *p* = 0.029). Both relationships were positive, suggesting that more abundant and/or diverse populations of methanogens are associated with higher rates of gross methanogenesis. We did not identify any individual prokaryotic taxa that were significantly associated with gross methanogenesis after accounting for

environmental and sample covariance and adjusting for multiple comparisons. Soil pH was the only significant abiotic predictor of methanogenesis (Adj. $R^2 = 0.24$, $p = 0.023$), showing a positive relationship whereby values approaching neutral pH had higher rates of methanogenesis.

Hydrogenotrophic methanogenesis

The most pronounced difference in the predominance of the hydrogenotrophic pathway was between mineral soil wetlands and peatlands, where it accounted for 30.7 ± 11.2 % and 76.7 ± 23.0 % of total methanogenesis, respectively (Fig. 1C). We identified a negative relationship between the DNA-inferred alpha diversity of the methanogens and the predominance of the hydrogenotrophic pathway (Shannon: Adj. $R^2 = 0.710$, $p < 0.001$ Fig. 1D, Table 2, Richness: Adj. $R^2 = 0.455$, $p = 0.002$). We also identified a negative relationship with the RNA-inferred alpha diversity of the prokaryotic community (Shannon: Adj. $R^2 = 0.609$, $p = 0.001$). The only abiotic variables that were significantly associated with hydrogenotrophic methanogenesis were a negative relationship with soil pH (Adj. $R^2=0.32$, $p < 0.01$), and a positive relationship with soil C (Adj. $R^2=0.341$, $p < 0.01$).

Methanotrophy processes

Gross methanotrophy

459 Average rates of gross CH_4 consumption (V_{max}) were higher in wetland sites
460 (including seasonal wetlands) (0.3 ± 0.05 μmol CH_4 g $soil^{-1}$ d^{-1}) than upland sites
461 (0.1 ± 0.02 μmol CH_4 g $soil^{-1}$ d^{-1}) (Fig. 2A, Kruskal-Wallis: Chi-squared = 6.85, df = 1,
462 p = 0.008), and significantly correlated with every abiotic variable measured. Gross
463 methanotrophy rates were negatively correlated with soil bulk density (Adj. R^2=
464 0.135, p =0.008). Note that the inverse of V_{max} is presented, i.e. higher uptake rates
465 are represented by higher values. Conversely gross methanotrophy rates were
466 positively correlated with soil moisture (Adj. R^2= 0.22, p < 0.001), soil C (Adj. R^2=
467 0.094, p =0.024), and soil N (Adj. R^2= 0.105, p =0.02). The only community attribute
468 that correlated with gross methanotrophy after accounting for variation due to soil
469 physicochemical variables was methanotroph abundance (i.e*. pmoA* copy number,
470 Adj. R^2=0.139, p=0.01, Fig. 2B, Table 2).
471
472 *High-affinity methanotrophy*
473 Of the four processes measured, high-affinity methanotrophy was the only
474 process to exhibit composition-based control involving the broader prokaryotic
475 community beyond known methanotrophs. Rates of high-affinity methane oxidation
476 varied considerably across our samples (range -9.15 to 2.08) (Fig. 2C) and were
477 higher in upland sites relative to wetland sites (including seasonal wetlands)
478 (Kruskal-Wallis: Chi-squared = 11.52, df = 1, p < 0.001). After accounting for sample
479 and environmental covariance, we identified 11 taxa in the DNA-inferred
480 community and 5 taxa in the RNA-inferred community whose relative abundance
481 significantly corresponded to high-affinity methanotrophy rates. The finest level of

taxonomic assignment for these taxa varied from order to genus. DNA-inferred taxa identified included members of the genera *Nitrosphaera* (Thaumarchaeota), *Roseiarcus* (Alphaproteobacteria), *Aminiphilus* (Synergistetes), and *Acidisoma* (Alphaproteobacteria), a member of the family Thermosporaceae (Actinobacteria), four members of Acidobacteria Group 2, and two members of Acidobacteria Group 6 (Supplementary Table 3). There was no ASV-level overlap with the RNA-inferred taxa that were identified. The RNA-inferred taxa included members of the genera *Beijerinckia* (Alphaproteobacteria), *Deferrisoma* (Deltaproteobacteria), *Nitrospirillum* (Alphaproteobacteria), the family Thermonosporaceae (Actinobacteria), and a member of Acidobacteria Group 1 (Supplementary Table 3).

We next ordinated the relative abundances of these taxa to reduce them to a single variable. The first principle component of both of these community subsets significantly correlated with high-affinity methanotrophy (RNA-inferred PC1 axis: Adj. $R^2 = 0.647$, $p < 0.001$, Fig. 2D, Table 2, DNA-inferred PC1 axis: Adj. $R^2 = 0.583$, $p < 0.001$). Lastly, we tested if high-affinity methanotrophy correlated with any other community attributes. After accounting for covariance structure, there were no other biotic correlates of high-affinity methanotrophy beyond composition-based variables.

DISCUSSION

The utility of microbial community attributes in predicting function

The utility of conventional microbial community measurements for predicting ecosystem functions has been recently called into question (Graham et

505 al., 2014; Inkpen et al., 2017; Louca et al., 2018; Rocca et al., 2014). Here we argue
506 that many of the difficulties associated with detecting community structure –
507 ecosystem function relationships could be overcome with careful consideration of
508 the community attributes chosen, the physiological scale of functional assessment,
509 and how environmental variation is controlled. In this study we have addressed
510 each of these concerns. Our work demonstrates 1) that there is a connection
511 between communities and ecosystem processes after accounting for variation due
512 to external factors, and 2) that the strength, as well as the nature, of the relationship
513 between communities and ecosystem function depends on the physiological scale of
514 the function.

515 Ecosystem function measurements face issues of physiological scale (Inkpen
516 et al., 2017), i.e. they represent the aggregate result of numerous underlying
517 processes. Detecting a causal link between community measurements and
518 ecosystem function thus becomes increasingly difficult for functions that include
519 inputs from multiple processes. This suggests that as one narrows the functional
520 scale to a level that more closely approximates individual physiologies, the strength
521 of the relationship with the community should increase. Our study supports this
522 hypothesis. For both methanogenesis- and methanotrophy-related processes, a
523 similar trend emerged; broader-scale functions had a weaker relationship with
524 community attributes than finer-scale functions. For instance, the best predictor of
525 gross methanogenesis was methanogen abundance (i.e. *mcrA* copy number),
526 explaining 47% of the variance, whereas hydrogenotrophic methanogensis – which
527 represents a finer physiological scale– showed the strongest association with

methanogen alpha diversity, explaining 71% of the variance (Table 2). For methanotrophy processes, gross methanotrophy was best predicted by methanotroph abundance (i.e. *pmoA* copy number), but this connection was quite weak and only explained ~14% of the variance. By contrast, much more of the variance in high-affinity methanotrophy could be explained by RNA-inferred community composition (~64%, Table 2). While our specific hypothesis only represents two processes for each type of function, our results provide support for the idea that the physiological scale of function is an important consideration for community structure-ecosystem function studies. Future work could extend the generality of our findings by assessing an even wider assortment of physiological scales (e.g. net CH_4 flux) or by extending this framework to other ecosystem functions (e.g. soil respiration).

Developing community variables that represent putative process controls

Communities can control processes in several distinct ways. It is therefore important that the variables we choose to represent communities reflect these types of control. One key finding from our work was that broader scale processes had distinctly different predictors than the underlying finer scale processes. Hence, several of the relationships uncovered by our work would have gone undetected had we focused solely on a single attribute. Both gross methanogenesis and methanotrophy were best predicted by abundance-based variables (i.e. *mcrA* and *pmoA* copy number, respectively), suggesting that these processes could be limited by the total number of individuals capable of performing them. Similar relationships

551 between abundance and methanotrophy/methanogenesis rates have been reported
552 (Freitag & Prosser, 2009; Ma et al., 2012), although a number of studies have
553 demonstrated no such relationship (see Rocca et al., 2014) – suggesting other
554 important controls. Hydrogenotrophic methanogenesis and high-affinity
555 methanotrophy – the finer-scale processes – by contrast were best predicted by
556 diversity and composition measures, respectively. Hydrogenotrophic predominance
557 was inversely related to methanogen diversity. One possible explanation for this
558 relationship is that more diverse methanogen consortia would be more likely to
559 contain other methanogen functional groups (*e.g.* acetoclastic or methylotrophic
560 taxa), which would thus affect the relative contribution of the hydrogenotrophic
561 pathway to gross methanogenesis.

562 The strongest – and only – biotic variables associated with high-affinity
563 methanotrophy were based on RNA- and DNA-inferred composition (i.e. taxon
564 identity), suggesting that trait differences among taxa could be especially important
565 for this process. Interestingly, none of the associated taxa we identified were known
566 methanotrophs. This insight highlights the importance of not restricting our search
567 to taxa that are canonically associated with methanotrophy. While there are issues
568 with the commensurability of taxonomic composition and ecosystem function
569 (Inkpen et al., 2017), these taxa could still be causally linked to high-affinity
570 methanotrophy in at least two ways. First, at least some could be non-canonical
571 methanotrophs that are consuming CH_4. Even though the ability to consume CH_4 is
572 conserved phylogenetically (Martiny et al., 2013), canonical methanotrophs (as well
573 as high affinity methanotrophs) are nevertheless polyphyletic (Knief, 2015),

574 suggesting that methanotrophy has evolved multiple times or has been laterally
575 transferred. Another possibility is that these taxa influence methanotrophy through
576 ecological interactions with methanotrophs (e.g. competition or facilitation). A final
577 possibility is that these taxa are simply a marker for high-affinity methanotrophy,
578 co-varying with a variable that is causally linked. One of the DNA-inferred taxa
579 identified, *Nitrosphaera sp.* (Thaumarchaeota), is a known ammonia oxidizer.
580 Ammonia monooxygenase and methane monooxygenase are phylogenetically
581 related (Holmes, Costello, Lidstrom, & Murrell, 1995), and it has been suggested that
582 ammonia oxidizers may be able to bind CH_4 (Arp, Sayavedra-Soto, & Hommes, 2002;
583 Ross & Rosenzweig, 2017). Another taxon, *Roseiarcus* (Alphaproteobacteria), is
584 closely related to alphaproteobacterial methanotrophs and has been isolated from a
585 methanotrophic consortium enriched from *Sphagnum* peat (Kulichevskaya,
586 Danilova, Tereshina, Kevbrin, & Dedysh, 2014). Thus, *Roseiarcus* could be either
587 directly consuming CH_4, or interacting with methanotrophs. Two of the RNA-
588 inferred taxa, *Nitrospirillum* and *Beijerinckia* (both Alphaproteobacteria), are
589 diazotrophs and could thus influence methanotroph activity by fixing atmospheric
590 N_2. The influence of other correlated taxa on methanotrophy is less clear, but
591 competition for soil nutrients/O_2, facilitation, or simply acting as a marker remain
592 possibilities.

593 While we attempted to equally represent each attribute in our modeling,
594 activity-based attributes were under-represented in this study. Transcriptional
595 levels of *pmoA* and *mcrA* have been used as proxies for methanotroph/methanogen
596 activity in a variety of studies (Chen, Dumont, Cébron, & Murrell, 2007; Freitag &

Prosser, 2009; Freitag et al., 2010). While we successfully quantified *pmoA* transcriptional activity, our attempt to do so for *mcrA* was problematic. This was not likely due to a lack of activity of methanogens (since CH_4 was continuously produced throughout the experiment), but rather due to PCR inhibitors such as humic substances present in organic-rich soils. We took our community inference methods a step further than many other studies by using both RNA- and DNA-based 16S rRNA profiling. It has been established that the relative abundances of taxa in the RNA-inferred community should not be used as a direct assessment of activity levels (Papp, Mau, et al., 2018; Papp, Hungate, et al., 2018). However, it is likely that the RNA-inferred community is at least enriched for the active fraction of soil taxa relative to the DNA-inferred community (which could contain higher proportions of inactive, dormant, or dead individuals) (Lennon & Jones, 2011). Thus, by including RNA-based community inference we were able to use an additional window onto the community that could have functional relevance.

Gaining a better understanding of tropical CH_4 dynamics

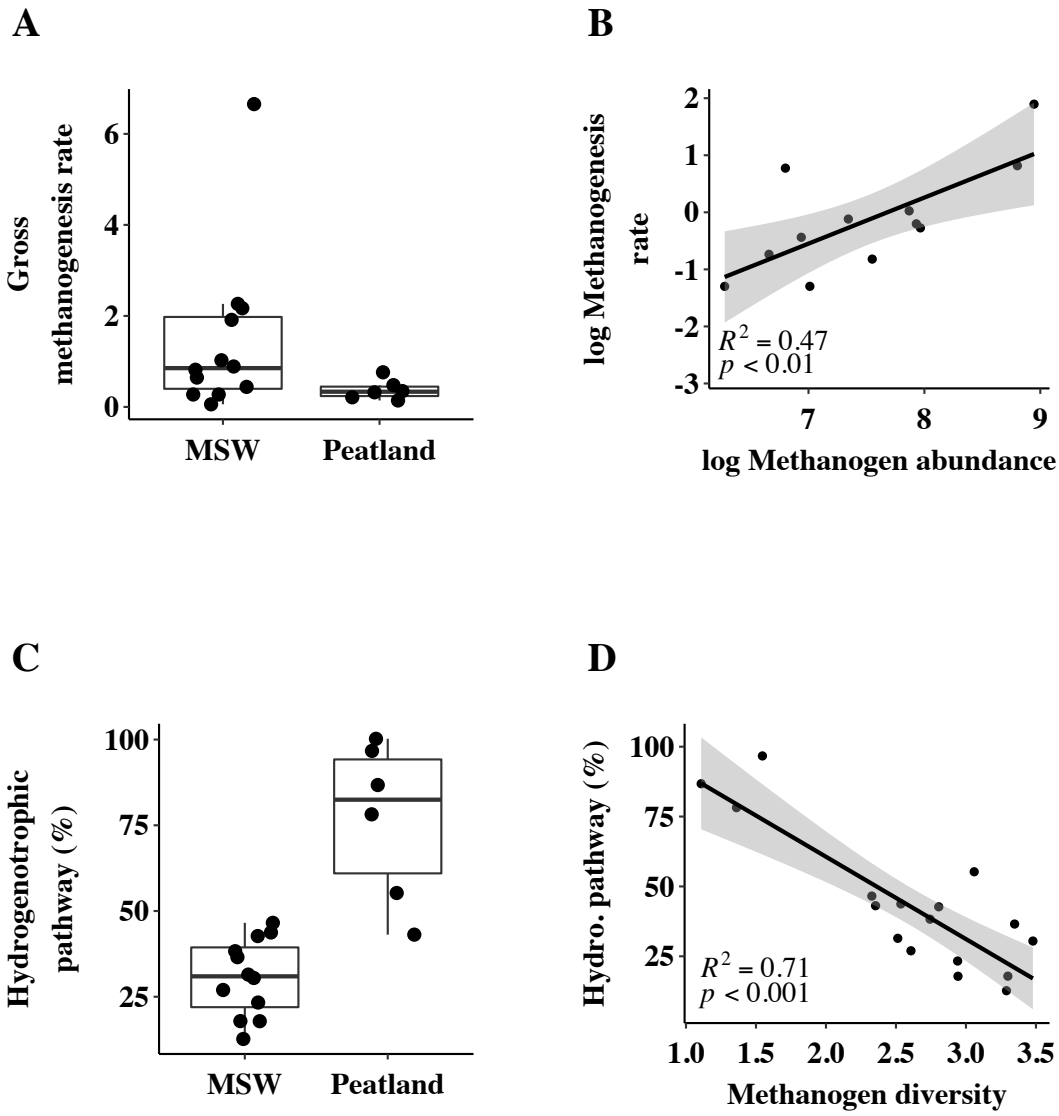
Despite the established importance of tropical ecosystems as global CH_4 sources (Kirschke et al., 2013; Sjögersten et al., 2014), our understanding of the ecological controls over CH_4-cycling throughout these regions remains limited (Bridgham et al., 2013). The majority of studies aimed at identifying controls on CH_4-cycling dynamics have been performed in higher latitude ecosystems such as northern peatlands (Bridgham et al., 2013). The contribution of biotic variables to CH_4-cycling dynamics may be a more important determinant of variation across

620 tropical environments relative to higher latitudes. Methanogen population growth
621 rates are slow because all methanogenic pathways have very low thermodynamic
622 yields that are often barely above the threshold for growth under *in-situ* conditions
623 (Conrad, 1999; Megonigal, Mines, & Visscher, 2004). Thus, methanogens inhabiting
624 high latitude environments must recover each season from freeze-thaw events and
625 will exhibit low levels of activity/growth until warmer temperatures are reached. In
626 tropical ecosystems where prolonged freezing conditions are essentially absent,
627 trait-level distributions of CH_4-cycling taxa could play a stronger role regulating
628 spatial differences in CH_4 dynamics. While our study was not designed to compare
629 differences between high- and low-latitude ecosystems, an interesting trend that
630 emerged from our results was that abiotic variables tended to be less predictive of
631 CH_4 cycling relative to biotic variables (with the exception of gross methanotrophy).
632 Importantly, our laboratory incubations deliberately downplayed the influence of
633 several key environmental factors (*e.g.* temperature fluctuations or rainfall events)
634 that could influence CH_4-cycling rates. Nevertheless, most of our environmental
635 variables were independent from our incubation conditions (*e.g.* %C, %N, pH, soil
636 bulk density), and each only explained a relatively small fraction of the variance.
637 Thus, an important outstanding question is whether the strength and nature of
638 biotic controls on CH_4-cycling dynamics differs between high- and low-latitude
639 ecosystems.
640
641 *Conclusion*

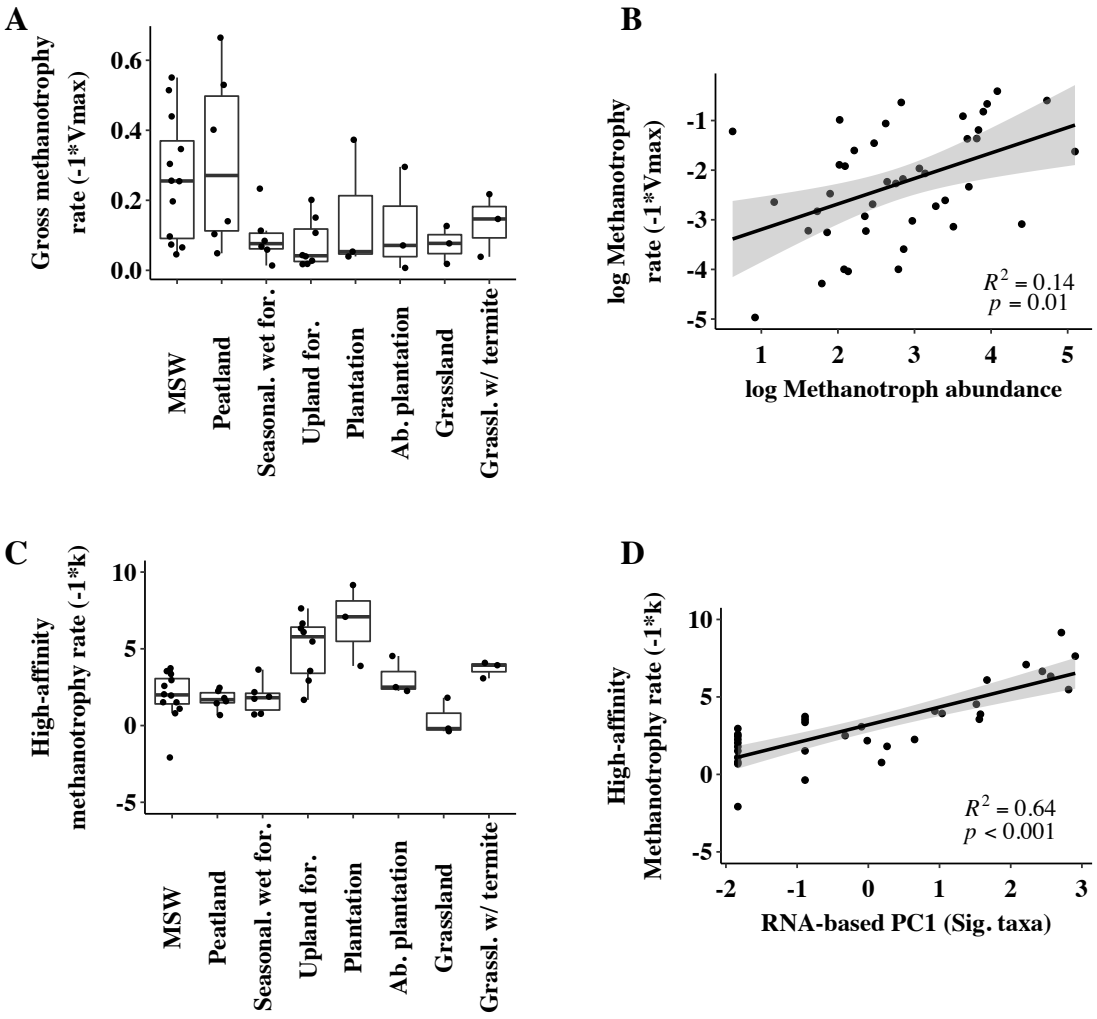
642 How communities map onto ecosystem function is a widely debated topic.
643 Central to this debate are issues related to the physiological scale of function, the
644 identification of useful community attributes, and accounting for the underlying
645 influence of the environment. Our study provides two unique perspectives to this
646 debate. First, we suggest that broad definitions of function could be obscuring the
647 connections with community attributes. Secondly, by compiling community
648 attributes according to their putative role in controlling processes, we show that
649 finer scale processes may face controls that differ from those of the coarser
650 processes to which they contribute. Collectively, these findings demonstrate the
651 importance of carefully defining the physiological scale of ecosystem function and
652 performing community measurements that represent putative controls on
653 ecosystem processes.
654
655 ACKNOWLEDGEMENTS
656 We thank the Government of Gabon, Centre National de la Recherche
657 Scientifique et Technologique for permission (Permit No
658 AR0035/14/MESRS/CENAREST/CG/CST/CSAR) to conduct this study. We thank F.
659 Bivigou, H. Memiaghe, L. Tchignoumba, E. Tobi, I. Akendengue for their help
660 collecting samples. The University of Oregon, the Gabon-Oregon Transnational
661 Center on Environment and Development, the Smithsonian Conservation Biology
662 Institute, and Shell Gabon provided financial and logistical support. BB and KM are
663 grateful for support by the National Science Foundation – Dimensions of
664 Biodiversity program (DEB 14422214), and SB and AH are grateful for support by

665 the Office of Biological and Environmental Research, Terrestrial Ecosystem Science
666 Program, U.S. Department of Energy (DE-SC0008092 and DE-39SC0012088).
667
668
669
670
671
672
673
674
675
676
677
678
679
680
681
682
683
684
685
686
687

688 FIGURES AND TABLES



689
690 **Fig. 1**: The rates and biotic drivers of methanogenesis-related processes across
691 Congo Basin wetlands. A) Methanogenesis rate (μmol CH_4 g $soil^{-1}$ d^{-1}) across the two
692 wetland habitats. MSW = mineral soil wetland. B) The log_2 methanogenesis rate is
693 best predicted by log_2 methanogen abundance (qPCR of *mcrA* gene). C) The
694 predominance (%) of the hydrogenotrophic pathway in gross methanogenesis
695 across wetland habitats. D) Hydrogenotrophic pathway predominance is inversely
696 related to the alpha diversity (Shannon index) of the methanogen community. Each
697 point represents an individual soil sample. Gray bands represent 95% confidence
698 intervals of the linear model.
699



700

701 **Fig. 2** The rates and biotic drivers of methanotrophy-related processes across
702 Congo Basin habitats. Note the inverse of V_{max} and k and presented so that higher
703 positive values correspond to higher rates of uptake. A) Gross methanotrophy rate
704 (-1* V_{max}) across habitats. MSW = mineral soil wetland, Seasonal. Wet for. =
705 seasonally inundated forest, Upland for. = upland forest, Ab. plantation = abandoned
706 plantation, Grassland w/ termite = grassland habitat dominated by termite mounds.
707 B) Gross methanotrophy rate is positively related to the log_2 abundance of
708 methanotrophs (*pmoA* gene copy number). C) High-affinity methanotrophy rate
709 across habitats. D) High-affinity methanotrophy rates are best predicted by the first
710 principle component (PC1) of a subset of significantly associated taxa (Sig. taxa)
711 identified in the RNA-inferred prokaryotic community. Gray bands represent 95%
712 confidence intervals of the linear model.
713

714

715 **Table 1:** The community attributes calculated in this study used to identify putative
716 community controls on process rates.

Attribute	Variables
Abundance	qPCR (of *mcrA* & *pmoA* genes)
	Relative abundance (in DNA-inferred prokaryotic community) of methanogens/methanotrophs
Activity	cDNA qPCR (pmoA)
	Relative abundance (in RNA-inferred prokaryotic community) of methanogens/methanotrophs
Composition	Matrix of 16S-based prokaryotic community inferred from RNA
	Matrix of 16S-based prokaryotic community (DNA-inferred)
	Matrix of methanogen/methanotroph taxa subsetted from 16S-based matrix (DNA-inferred)
	Species (ASV) richness of RNA/DNA-inferred prokaryotic community
Diversity	ASV richness of methanogen/methanotroph community subsets (RNA & DNA)
	Shannon (ASV) diversity of RNA/DNA-inferred prokaryotic community
	Shannon diversity of methanogen/methanotroph community subsets (RNA & DNA)

717

718

719

720

721

722

723

724

725

726

727

728 **Table 2:** Narrow scale processes have a higher association with community
729 attributes. Process assessment scale: whether a process assessment is the aggregate
730 result of several processes (broad) or only one (fine). Adj. R^2 = the proportion of the
731 variance explained by the attribute.
732

	Physiological scale of process	Best fit community attribute	Adj. R^2	*P* value
Methanotrophy processes				
Gross Methanotrophy (V_{max})	Broad	Abundance (*pmoA* copy)	0.14	0.01
High affinity methanotrophy (k)	Fine	Composition (RNA-inferred)	0.64	< 0.001
Methanogenesis processes				
Gross Methanogenesis (μmol CH_4 g $soil^{-1}$ d^{-1})	Broad	Abundance (*mcrA* copy)	0.47	0.008
Hydrogenotrophic methanogenesis (% gross CH_4 production)	Fine	Diversity (methanogens)	0.79	< 0.001

733

734 DATA ACCESSIBILITY

735 Sequence data, sample meta data, biogeochemical data, ASV community matrices

736 and R script for analysis will be made available upon acceptance of publication.

737

738 AUTHOR CONTRIBUTIONS

739 KM, AH, SB and BB designed and performed research. KM, AH, AK, and AM analyzed

740 data. KM wrote the paper with contributions from all authors.

741

742

743

744

745

REFERENCES

Arp, D. J., Sayavedra-Soto, L. A., & Hommes, N. G. (2002). Molecular biology and biochemistry of ammonia oxidation by Nitrosomonas europaea. *Archives of Microbiology*, *178*(4), 250–255. http://doi.org/10.1007/s00203-002-0452-0

Baldrian, P., Kolařík, M., Stursová, M., Kopecký, J., Valášková, V., Větrovský, T., … Voříšková, J. (2012). Active and total microbial communities in forest soil are largely different and highly stratified during decomposition. *The ISME Journal*, *6*(2), 248–58. http://doi.org/10.1038/ismej.2011.95

Bannar-Martin, K. H., Kremer, C. T., Ernest, S. M., Leibold, M. A., Auge, H., Chase, J., … Supp, S. R. (2018). Integrating community assembly and biodiversity to better understand ecosystem function: the Community Assembly and the Functioning of Ecosystems (CAFE) approach. *Ecology Letters*, *21*, 167–180. http://doi.org/10.1111/ele.12895

Bier, R. L., Bernhardt, E. S., Boot, C. M., Graham, E. B., Hall, E. K., Lennon, J. T., … Wallenstein, M. D. (2015). Linking microbial community structure and microbial processes: An empirical and conceptual overview. *FEMS Microbiology Ecology*, *91*(10), 1–11. http://doi.org/10.1093/femsec/fiv113

Bodelier, P. L. E., Meima-Franke, M., Hordijk, C. a, Steenbergh, A. K., Hefting, M. M., Bodrossy, L., … Seifert, J. (2013). Microbial minorities modulate methane consumption through niche partitioning. *The ISME Journal*, *7*(11), 2214–28. http://doi.org/10.1038/ismej.2013.99

Bodelier, P. L. E., Roslev, P., Henckel, T., & Frenzel, P. (2000). Stimulation by ammonium-based fertilizers of methane oxidation in soil around rice roots. *Nature*, *403*, 421–424.

Bourne, D. G., Donald, I. A. N. R. M. C., & Murrell, J. C. (2001). Comparison of pmoA PCR Primer Sets as Tools for Investigating Methanotroph Diversity in Three Danish Soils. *Applied and Environmental Microbiology*, *67*(9), 3802–3809. http://doi.org/10.1128/AEM.67.9.3802

Bridgham, S. D., Cadillo-Quiroz, H., Keller, J. K., & Zhuang, Q. (2013). Methane emissions from wetlands: Biogeochemical, microbial, and modeling perspectives from local to global scales. *Global Change Biology*, *19*(5), 1325–1346. http://doi.org/10.1111/gcb.12131

Bridgham, S. D., & Ye, R. (2013). Organic Matter Mineralization and Decomposition. In R. D. DeLaune, K. R. Reddy, C. J. Richardson, & J. P. Megonigal (Eds.), *Methods in Biogeochemistry of Wetlands* (pp. 385–406). Madison, WI: SSSA.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*, 581. Retrieved from http://dx.doi.org/10.1038/nmeth.3869

Caporaso, J. G., Kuczynski, J., Stombaugh, J., Bittinger, K., Bushman, F. D., Costello, E. K., … Knight, R. (2010). QIIME allows analysis of high- throughput community sequencing data Intensity normalization improves color calling in SOLiD sequencing. *Nature Methods*, *7*(5), 335–336. http://doi.org/10.1038/NMETH.F.303

Caporaso, J. G., Lauber, C. L., Walters, W. A., Berg-lyons, D., Lozupone, C. A.,

Turnbaugh, P. J., … Knight, R. (2011). Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. *Proceedings of the National Academy of Sciences*, *108*, 4516–4522. http://doi.org/10.1073/pnas.1000080107/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1000080107

Chen, Y., Dumont, M. G., Cébron, A., & Murrell, J. C. (2007). Identification of active methanotrophs in a landfill cover soil through detection of expression of 16S rRNA and functional genes. *Environmental Microbiology*, *9*(11), 2855–69. http://doi.org/10.1111/j.1462-2920.2007.01401.x

Cole, J. R., Wang, Q., Fish, J. A., Chai, B., McGarrell, D. M., Sun, Y., … Tiedje, J. M. (2014). Ribosomal Database Project: data and tools for high throughput rRNA analysis. *Nucleic Acids Research*, *42*(D1), D633–D642. http://doi.org/10.1093/nar/gkt1244

Conrad, R. (1999). Contribution of hydrogen to methane production and control of hydrogen concentrations in methanogenic soils and sediments. *FEMS Microbiology Ecology*, *28*(3), 193–202. http://doi.org/10.1016/S0168-6496(98)00086-5

Delmas, R. A., Tathy, J. P., & Cros, B. (1992). Atmospheric methane budget in Africa. *Journal of Atmospheric Chemistry*, *14*(1), 395–409. http://doi.org/10.1007/BF00115247

Delmas, R., Servant, J., Tathy, J., Cros, B., & Labat, M. (1992). Sources and sinks of methane and carbone dioxide exchanges in mountain forest in Equatorial Africa. *Journal of Geophysical Research*, *97*(90), 6169–6179.

Fadrosh, D. W., Ma, B., Gajer, P., Sengamalay, N., Ott, S., Brotman, R. M., & Ravel, J. (2014). An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. *Microbiome*, *2*(6), 1–7.

Freitag, T. E., & Prosser, J. I. (2009). Correlation of methane production and functional gene transcriptional activity in a peat soil. *Applied and Environmental Microbiology*, *75*(21), 6679–87. http://doi.org/10.1128/AEM.01021-09

Freitag, T. E., Toet, S., Ineson, P., & Prosser, J. I. (2010). Links between methane flux and transcriptional activities of methanogens and methane oxidizers in a blanket peat bog. *FEMS Microbiology Ecology*, *73*, 157–165. http://doi.org/10.1111/j.1574-6941.2010.00871.x

Garcia, J. L., Patel, B. K., & Ollivier, B. (2000). Taxonomic, phylogenetic, and ecological diversity of methanogenic Archaea. *Anaerobe*, *6*(4), 205–26. http://doi.org/10.1006/anae.2000.0345

Gavlak, R., Horneck, D., Miller, R. O., & Kotuby-Amarcher, J. (2003). Soil, Plant, and Water Reference Methods for the Western Region. *Western Region Extension Publication*, *WREP-125*.

Graham, E. B., Wieder, W. R., Leff, J. W., Weintraub, S. R., Townsend, A. R., Cleveland, C. C., … Nemergut, D. R. (2014). Do we need to understand microbial communities to predict ecosystem function ? A comparison of statistical models of nitrogen cycling processes. *Soil Biology and Biochemistry*, *68*, 279–282. http://doi.org/10.1016/j.soilbio.2013.08.023

Ho, A., Kerckhof, F.-M., Luke, C., Reim, A., Krause, S., Boon, N., & Bodelier, P. L. E. (2013). Conceptualizing functional traits and ecological characteristics of methane-oxidizing bacteria as life strategies. *Environmental Microbiology*

Reports, *5*(3), 335–45. http://doi.org/10.1111/j.1758-2229.2012.00370.x

Holmes, A. J., Costello, A., Lidstrom, M. E., & Murrell, J. C. (1995). Evidence that particulate methane monooxygenase and ammonia monooxygenase may be evolutionarily related. *FEMS Microbiology Letters*, *132*, 203–208.

Inkpen, S. A., Douglas, G. M., Brunet, T. D. P., Leuschen, K., Doolittle, W. F., & Langille, M. G. I. (2017). The coupling of taxonomy and function in microbiomes. *Biology & Philosophy*. http://doi.org/10.1007/s10539-017-9602-2

Jones, S. E., & Lennon, J. T. (2010). Dormancy contributes to the maintenance of microbial diversity. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(13), 5881–6. http://doi.org/10.1073/pnas.0912765107

Kamke, J., Taylor, M. W., & Schmitt, S. (2010). Activity profiles for marine sponge-associated bacteria obtained by 16S rRNA vs 16S rRNA gene comparisons. *The ISME Journal*, *4*(4), 498–508. http://doi.org/10.1038/ismej.2009.143

Keller, J., & Bridgham, S. (2007). Pathways of anaerobic carbon cycling across an ombrotrophic-minerotrophic peatland gradient. *Limnology and Oceanography*, *52*(1), 96–107. http://doi.org/10.4319/lo.2007.52.1.0096

Kirschke, S., Bousquet, P., Ciais, P., Saunois, M., Canadell, J. G., Dlugokencky, E. J., … Zeng, G. (2013). Three decades of global methane sources and sinks. *Nature Geoscience*, *6*(10), 813–823. Retrieved from http://dx.doi.org/10.1038/ngeo1955

Knief, C. (2015). Diversity and habitat preferences of cultivated and uncultivated aerobic methanotrophic bacteria evaluated based on pmoA as molecular marker. *Frontiers in Microbiology*, *6*, 1–38. http://doi.org/10.3389/fmicb.2015.01346

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

Kozich, J. J., Westcott, S. L., Baxter, N. T., Highlander, S. K., & Schloss, P. D. (2013). Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. *Applied and Environmental Microbiology*, *79*(17), 5112–20. http://doi.org/10.1128/AEM.01043-13

Kulichevskaya, I. S., Danilova, O. V, Tereshina, V. M., Kevbrin, V. V, & Dedysh, S. N. (2014). Descriptions of Roseiarcus fermentans gen. nov., sp. nov., a bacteriochlorophyll a-containing fermentative bacterium related phylogenetically to alphaproteobacterial methanotrophs, and of the family Roseiarcaceae fam. nov. *International Journal of Systematic and Evolutionary Microbiology*, *64*(8), 2558–2565. https://ijs.microbiologyresearch.org/content/journal/ijsem/10.1099/ijs.0.064576-0

Lee, M. E., Alonso, A., Dallmeier, F., Campbell, P., & Pauwels, O. S. G. (2006). The Gamba Complex of Protected Areas : An Illustration of Gabon's Biodiversity. *Bulletin of the Biological Society of Washington*, (12), 229–242.

Legendre, P. (1993). Spatial autocorrelation: trouble or new paradigm? *Ecology*, *74*(6), 1659–1673.

Lennon, J. T., & Jones, S. E. (2011). Microbial seed banks: the ecological and evolutionary implications of dormancy. *Nature Reviews Microbiology*, *9*(2), 119–30. http://doi.org/10.1038/nrmicro2504

Loreau, M., Naeem, S., Inchausti, P., Bengtsson, J., Grime, J. P., Hector, A., … Wardle, D. A. (2001). Biodiversity and Ecosystem Functioning: Current Knowledge and Future Challenges. *Science*, *294*(5543), 804 LP-808. Retrieved from http://science.sciencemag.org/content/294/5543/804.abstract

Louca, S., Polz, M. F., Mazel, F., Albright, M. B. N., Huber, J. A., Connor, M. I. O., … Parfrey, L. W. (2018). Function and functional redundancy in microbial systems. *Nature Ecology & Evolution*. http://doi.org/10.1038/s41559-018-0519-1

Ma, K., Conrad, R., & Lu, Y. (2012). Responses of methanogen mcrA genes and their transcripts to an alternate dry/wet cycle of paddy field soil. *Applied and Environmental Microbiology*, *78*(2), 445–54. http://doi.org/10.1128/AEM.06934-11

MacDonald, J. A., Jeeva, D., Eggleton, P., Davies, R., Bignell, D. E., Fowler, D., … Maryati, M. (1999). The effect of termite biomass and anthropogenic disturbance on the CH4 budgets of tropical forests in Cameroon and Borneo. *Global Change Biology*, *5*(8), 869–879.

Martiny, A. C., Treseder, K., & Pusch, G. (2013). Phylogenetic conservatism of functional traits in microorganisms. *The ISME Journal*, *7*(4), 830–8. http://doi.org/10.1038/ismej.2012.160

McCalley, C. K., Woodcroft, B. J., Hodgkins, S. B., Wehr, R. a., Kim, E.-H., Mondav, R., … Saleska, S. R. (2014). Methane dynamics regulated by microbial community response to permafrost thaw. *Nature*, *514*(7523), 478–481. http://doi.org/10.1038/nature13798

Megonigal, J. P., Mines, M. E., & Visscher, P. T. (2004). Anaerobic Metabolism: Linkages to Trace Gases and Aerobic Processes. In *Biogeochemistry* (Vol. 8, pp. 350–362). Gulf Professional Publishing.

Nazaries, L., Murrell, J. C., Millard, P., Baggs, L., & Singh, B. K. (2013). Methane, microbes and models: fundamental understanding of the soil methane cycle for future predictions. *Environmental Microbiology*, *15*(9), 2395–417. http://doi.org/10.1111/1462-2920.12149

Nazaries, L., Pan, Y., Bodrossy, L., Baggs, E. M., Millard, P., Murrell, J. C., & Singh, B. K. (2013). Evidence of microbial regulation of biogeochemical cycles from a study on methane flux and land use change. *Applied and Environmental Microbiology*, *79*(13), 4031–40. http://doi.org/10.1128/AEM.00095-13

Oksanen, J., Blanchet, F. G., Roeland, K., Legendre, P., Minchin, P., O'Hara, R. B., … Wagner, H. (2015). vegan: Community ecology package. Retrieved from http://cran.r-project.org

Papp, K., Hungate, B. A., & Schwartz, E. (2018). Microbial rRNA Synthesis and Growth Compared through Quantitative Stable Isotope Probing with H218O. *Applied and Environmental Microbiology*, *84*(8), 1–11.

Papp, K., Mau, R. L., Hayer, M., Koch, B. J., Hungate, B. A., & Schwartz, E. (2018). Quantitative stable isotope probing with H218O reveals that most bacterial taxa in soil synthesize new ribosomal RNA. *The ISME Journal*, 18–20.

http://doi.org/10.1038/s41396-018-0233-7

R Core Team. (2018). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for statistical computing. Retrieved from http://cran.r-project.org

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

Rocca, J. D., Hall, E. K., Lennon, J. T., Evans, S. E., Waldrop, M. P., Cotner, J. B., … Wallenstein, M. D. (2014). Relationships between protein-encoding gene abundance and corresponding process are commonly assumed yet rarely observed. *The ISME Journal*, *9*(8), 1693–1699. http://doi.org/10.1038/ismej.2014.252

Ross, M. O., & Rosenzweig, A. C. (2017). A tale of two methane monooxygenases. *Journal of Biological Inorganic Chemistry*, *22*(2–3), 307–319. http://doi.org/10.1007/s00775-016-1419-y

Ruijter, J. M., Ramakers, C., Hoogaars, W. M. H., Karlen, Y., Bakker, O., van den Hoff, M. J. B., & Moorman, a F. M. (2009). Amplification efficiency: linking baseline and bias in the analysis of quantitative PCR data. *Nucleic Acids Research*, *37*(6), e45. http://doi.org/10.1093/nar/gkp045

Schimel, J. P. (1995). Ecosystem Consequences of Microbial Diversity and Community Structure. In F. S. Chapin III & C. Körner (Eds.), *Arctic and Alpine Biodiversity: Patterns, Causes and Ecosystem Consequences* (pp. 239–254). Berlin: Springer-Verlag.

Schimel, J. P., & Gulledge, J. (1998). Microbial community structure and global trace gases. *Global Change Biology*, *4*, 745–758.

Schmieder, R., & Edwards, R. (2011). Quality control and preprocessing of metagenomic datasets. *Bioinformatics*, *27*(6), 863–864. http://doi.org/10.1093/bioinformatics/btr026

Schnyder, E., Bodelier, P., Hartmann, M., Henneberger, R., & Niklaus, P. (2018). Positive diversity-functioning relationships in model communities of methanotrophic bacteria. *Ecology*, *99*(3), 714–723. http://doi.org/10.1002/ecy.2138

Sierocinski, P., Bayer, F., Melia, G. Y., Großkopf, T., Alston, M., Swarbreck, D., … Buckling, A. (2018). Biodiversity – function relationships in methanogenic communities. *Molecular Ecology*, *27*, 4641–4651. http://doi.org/10.1111/mec.14895

Sierocinski, P., Milferstedt, K., Bayer, F., Großkopf, T., Alston, M., Bastkowski, S., … Buckling, A. (2017). A Single Community Dominates Structure and Function of a Mixture of Multiple Methanogenic. *Current Biology*, 3390–3395.

Sjögersten, S., Black, C. R., Evers, S., Hoyos-Santillan, J., Wright, E. L., & Turner, B. (2014). Tropical wetlands: A missing link in the global carbon cycle? *Global Biogeochemical Cycles*, *28*, 1371–1386. http://doi.org/10.1002/2014GB004844.Received

Steinberg, L. M., & Regan, J. M. (2008). Phylogenetic comparison of the methanogenic communities from an acidic, oligotrophic fen and an anaerobic digester treating

975 municipal wastewater sludge. *Applied and Environmental Microbiology*, *74*(21),
976 6663–71. http://doi.org/10.1128/AEM.00553-08
977 Tathy, J. P., Cros, B., Delmas, R. A., Marenco, A., Servant, J., & Labat, M. (1992).
978 Methane emission from flooded forest in central Africa. *Journal of Geophysical*
979 *Research: Atmospheres*, *97*(D6), 6159–6168.
980 http://doi.org/10.1029/90JD02555
981 Tilman, D., & Downing, J. A. (1994). Biodiversity and stability in grasslands. *Nature*,
982 *367*, 363–365.
983 Tilman, D., Wedin, D., & Knops, J. (1996). Productivity and sustainability influenced
984 by biodiversity in grassland ecosystems. *Nature*, *379*, 718. Retrieved from
985 http://dx.doi.org/10.1038/379718a0
986 Wang, Q., Garrity, G. M., Tiedje, J. M., & Cole, J. R. (2007). Naive Bayesian Classifier for
987 Rapid Assignment of rRNA Sequences into the New Bacterial Taxonomy.
988 *Applied and Environmental Microbiology*, *73*(16), 5261–5267.
989 http://doi.org/10.1128/AEM.00062-07
990 White, F. (1979). The Guineo-Congolian Region and Its Relationships to Other
991 Phytochoria. *Bulletin Du Jardin Botanique National de Belgique / Bulletin van de*
992 *National Plantentuin van Belgie*, *49*(1/2), 11–55.
993 http://doi.org/10.2307/3667815
994 Wickham, H. (2009). *ggplot2: elegant graphics for data analysis*. Springer Science &
995 Business Media.
996 Zhang, J., Kobert, K., Flouri, T., & Stamatakis, A. (2014). PEAR: a fast and accurate
997 Illumina Paired-End reAd mergeR. *Bioinformatics*, *30*(5), 614–620.
998 http://doi.org/10.1093/bioinformatics/btt593
999