INTRODUCTION

Consistent with the large body of work on plant communities (Grime, 1997; Hector et al., 1999; Hooper, Adair, & Cardinale, 2012; Nielsen, Ayres, Wall, & Bardgett, 2011; Tilman, 1997), microbial diversity can have a positive role in a range of community functions, including aerobic respiration, litter decomposition and plant growth (Bell, Newman, Silverman, Turner, & Lilley, 2005; Delgado-Baquerizo et al., 2016; Handa et al., 2014; Philippot et al., 2013; Wagg, Bender, Widmer, & van der Heijden, 2014). Strongly positive diversity–function relationships imply little functional redundancy of community members, and hence, loss of diversity resulting from environmental change may have considerable impact on community function (Jax, 2005; Loreau, 1998). One of the key microbial ecosystem functions where the role of diversity has not been experimentally investigated is methanogenesis: methane production resulting from the anaerobic conversion of H₂, CO₂ and short chain fatty acids by archaean methanogens (Ferry, 2012). Methane is both a potent greenhouse gas and a renewable resource from organic waste; therefore, determining causal links between microbial community diversity, composition and methanogenesis is important.
Research investigating the links between methanogenesis and microbial diversity has been correlational. Studies of methanogenesis in natural soil communities have reported positive correlations between methane production (from an incubated soil sample) and the diversity of both methanogens and the total bacterial/archaeal communities (Wagner, Zona, Oechel, & Lipson, 2017; Yavitt, Yashiro, Cadillo-Quiroz, & Zinder, 2012). However, any conclusions are potentially confounded by other environmental variables, such as pH, that can have a major role on community structure (Fierer et al., 2012; Hesse et al., 2018) and methanogenesis (Wagner et al., 2017). Other studies have focussed on correlations between community structure and methanogenesis under "common garden” laboratory conditions, where environmental factors are better controlled. The largest of these, involving 150 samples (Venkiteshwaran et al., 2017), showed no relationship between diversity and function, but in this case, the composition of communities differed in many ways in addition to diversity, and biomass was not controlled for.

As a consequence, there is a clear need to conduct manipulative experiments where causal links between diversity and methanogenesis can be determined. Manipulating diversity of methanogenic communities is non-trivial: They are typically very complex, consisting of varied taxa, most of which cannot be easily grown in pure culture or even cultivated at all. This makes the factorial manipulation of diversity at ecologically relevant levels almost impossible. Diversity can, however, be manipulated by dilution (Hernandez-Raquet, Durand, Braun, Cravo-Laureau, & Godon, 2013; Philippot et al., 2013; Salonius, 1981), which necessarily results in the loss of rare species relative to common species.

Here, we conduct such a dilution manipulation across six orders of magnitude on a methanogenic ancestral community obtained by mixing twelve separate communities. We have previously shown that mixing multiple communities maximizes the function and diversity in the mix (Sierocinski et al., 2017), thus using the mix maximized our chance of generating a highly functional community in the process. We allowed the biomass of the diluted cultures to become re-established over months in laboratory reactors and then densities equalized between treatments. Methane production was subsequently measured over six weeks in laboratory anaerobic digesters. In an attempt to assess the importance of diversity of rare species relative to other differences in community composition, we also investigated correlations between diversity and methanogenesis in natural communities isolated from a range of industrial anaerobic digesters and associated feedstock environments (sewage, silage, slurry, etc) over eight weeks. A number of studies suggest that novel propagation conditions impose selection pressures can result in large changes in the composition of methanogenic communities (De Vrieze et al., 2015; Mladenovska, Dabrowski, & Ahring, 2003; Regueiro et al., 2012; Vanwonterghem et al., 2014), and hence, we determined community composition at the start and end of the experiment.

2 | MATERIALS AND METHODS

2.1 | Natural communities

In order to use samples that varied in diversity and methane production, we collected six pseudo-pairs of anaerobic digester and feedstock samples. Anaerobic digester samples came from inside the fermentation tank, while feedstock samples were either acquired from the fermenter feedstock or fermenter seeding material (Table 1). The cultures were grown for eight weeks in 500-mL bottles (total volume with headspace: 600 mL, Duran) using Automated Methane Potential Test System (AMPTS, Bioprocess Control Sweden AB) to measure CO\textsubscript{2}-stripped biogas production. We confirmed that the resulting stripped gas was >95% methane by comparing the composition of the produced gas pre- and poststripping using GC-FID (Agilent, 7890A) and comparing these with a standard curve made using methane standard (Sigma). Each sample was replicated in four fermenters, two of them fed using a synthetic medium displaying a C:N ratio of 15:1 and the other two with 30:1 C:N ratio. We used C:N ratios of 15 and 30 because they were reported to be close to optimal values for slurry (Hills, 1979; Hills & Roberts, 1981) and

| AD Sample ID | Location | Feed | Temp. | Time since last seeding [months] | Paired sample ID | Feedstock type |
|--------------|----------|------|-------|---------------------------------|-----------------|----------------|
| AD1          | Farm     | 70% grass and maize silage; 30% food waste | 42–44°C | 14 | AD7 | Cow slurry |
| AD3          | Farm     | Maize; cow slurry; chicken manure | 45°C | 12 | AD4 | Maize; cow slurry; chicken manure |
| AD5          | Sewage   | Sewage sludge | 36°C | 12 | AD14 | Sewage sludge predigester |
| AD9          | Sewage   | Sewage slurry postdigester | 36°C | 60 | AD8 | Thickened sewage sludge |
| AD10         | Farm     | Food waste | 36°C | 18 | AD11 | Cow slurry |
| AD13         | Farm     | Maize/grass silage; cow slurry; chicken manure | 40°C | 5 | AD12 | Maize/grass silage; cow slurry; chicken manure |
wastewater (Rughoonundun, Mohee, & Holtzapple, 2012)-treating anaerobic digesters, respectively. Starting densities were equalized using 1×M9 salts (Na₂HPO₄•7H₂O, 12.8 g L⁻¹, KH₂PO₄, 15 g L⁻¹, NaCl, 2.5 g L⁻¹, NH₄Cl, 5.0 g L⁻¹) to 2 × 10⁶ [cells mL⁻¹] based on qPCR measurements of 16S RNA genes (see below). 300 g of each sample was used as an inoculum and fed weekly with 25 mL of defined medium that mimicked the composition of standard anaerobic digestor feed systems composed of slurry and plant matter: meat extract 111.1 g L⁻¹, cellulose 24.9 g L⁻¹, starch 9.8 g L⁻¹ glucose 0.89 g L⁻¹, xylose 3.55 g L⁻¹ for carbon-to-nitrogen ratio of 15:1 and meat extract 73.2 g L⁻¹, cellulose 35.5 g L⁻¹, starch 13.9 g L⁻¹ glucose 1.27 g L⁻¹, xylose 5.07 g L⁻¹ for C:N ratio of 30:1 (all Sigma). Additionally, before the start of the fermentation, 0.3 mL of 1,000× trace metal stock (1 g L⁻¹ FeCl₂•4H₂O, 0.5 g L⁻¹ MnCl₂•4H₂O, 0.3 g L⁻¹ CoCl₂•4H₂O, 0.2 g L⁻¹ ZnCl₂, 0.1 g L⁻¹ NiSO₄•6H₂O, 0.05 g L⁻¹ Na₂MoO₄•4H₂O, 0.02 g L⁻¹ H₂BO₃, 0.008 g L⁻¹ Na₂ WO₄•2H₂O, 0.006 g L⁻¹ Na₂ SeO₂•5H₂O, 0.002 g L⁻¹ CuCl₂•2H₂O) was added to each fermenter. The fermenters were run in two eight-week batches.

2.2 | Dilution experiment

Initial inoculum was diluted by putting 3 mL into 100-mL serum flasks with butyl rubber stoppers, containing 2.5 g of 15:1 C:N sugar mixture, 3 mL of 10x M9 salts (also see above), 21.47 g of sterile deionized water and 0.03 mL of 1,000× minerals solution (see above). The process has been serially repeated till the dilution of 10⁻⁶. Anaerobic conditions were ensured by filling the flasks with oxygen-free nitrogen, and 1 mg/L resazurin was added to the medium to identify possible oxygen contamination. Six flasks of 10-fold diluted culture were established and each independently serially diluted five times in ten-fold steps by transferring 3 mL to produce dilutions ranging from 10⁻¹ to 10⁻⁶. These dilutions have been incubated for three months at 35°C in order to regain the lost biomass. After that time, we measured the number of cells in each flask using optical density measurements at 600 nm (OD₆₀₀) to make sure that they have regrown to measurable values, therefore showing that the diluted communities were still functional. Consequently, we transferred the cultures to AMPTS II system at equal densities. Cultures were regrown to measurable values, therefore showing that the diluted communities were still functional. Consequently, we transferred

2.3 | DNA extractions

DNA was extracted using FastDNA™ SPIN Kit for Soil (MP) for the sequencing and Qiagen QIAamp DNA Stool Mini Kit for all the qPCR assays. The quality and quantity of the extractions were confirmed by 1% agarose gel electrophoresis and dsDNA BR (Qubit), respectively. We extracted DNA from one out of four replicates per community (a 15:1 C:N replicate) at the start and end of the experiment (pre- and postexperiment) involving the natural samples and from all samples at end of the experiment (postexperiment) in the dilution experiment.

2.4 | Real-time PCR assay

We used real-time PCR followed by dilution to standardize starting microbial densities in the natural communities, because OD₆₀₀ estimates of density would have been confounded by differences in the environments from which communities were sampled from. To ensure the method was accurate, we carried out a ten-fold dilution series, confirmed by plating of a control bacterium, *Pseudomonas fluorescens* SBW25. We then extracted DNA of each dilution using the QiAamp DNA Stool Mini Kit. The DNA was amplified by qPCR using 16S rRNA primers 338f and 518r (Øvreås & Torsvik, 1998). The extracted dilution series (Y = −3.359 × log₁₀X + 44.65; PCR efficiency = 98.5%; r² = 0.99) was compared to a curve of a DNA sample from slurry diluted after extraction (Y = −3.353 × log₁₀X + 13.52; PCR efficiency = 98.7%; r² = 1.0), indicating that the efficiency of the standard was comparable to the efficiency of the samples. PCR efficiency relates to the amplification per cycle efficiency, with 100% meaning doubling of DNA every cycle, the theoretical maximum.

For the postexperimental samples, we used a genomic DNA standard extracted from *P. fluorescens* for the Bacteria and *Halobacterium salinarum* for Archaea. The genome mass was calculated (Dolezel, Bartos, Voglmayr, & Greilhuber, 2003) and divided by the 16S gene copy number. DNA content (pg) = genome size (bp)/(0.978 × 10⁹) per copy number per gene of interest. The DNA quantity was measured using a Qubit dsDNA BR Assay Kit on a Qubit 2.0 Fluorometer, with the DNA diluted to concentrations containing 10⁶ 16S rRNA gene copies per μL and 10⁷ copies per μL for Archaea.

All pre-experiment samples were run on a Stratagene MX3005P thermal cycler with 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s finalized by a melt curve of 95°C for 1 min and 55°C ramping up to 95°C (15 s for each step). All postexperiment samples were run on an Applied Biosystems StepOnePlus thermal cycler 95°C (3 min) 40 cycles 95°C (5 s), 60°C (10 s) flowed by a melting curve of 95°C (15 s) 60°C ramping up to 95°C in steps of 0.3°C (15 s for each step). The primers (Øvreås & Torsvik, 1998) used to identify Bacteria were 16S rRNA gene libraries were constructed using primers designed for the Bacteria and *Pseudomonas fluorescens* for the Archaea. All pre-experiment samples were run on a Stratagene MX3005P thermal cycler with 95°C for 3 min, followed by 40 cycles of 95°C for 15 s, 60°C for 20 s finalized by a melt curve of 95°C for 1 min and 55°C ramping up to 95°C (15 s for each step). All postexperiment samples were run on an Applied Biosystems StepOnePlus thermal cycler 95°C (3 min) 40 cycles 95°C (5 s), 60°C (10 s) flowed by a melting curve of 95°C (15 s) 60°C ramping up to 95°C in steps of 0.3°C (15 s for each step). All samples were run in triplicates. The samples were compared to the standards using the software: MXPro MX3005P v4.10 Build 389 (Agilent) for the ancestral and stepone Software 2.3 for the descendant samples. Note slightly different methods were used because of a necessary change in equipment and that there were no direct comparisons between pre- and postexperiment samples.

2.5 | Amplicon library construction and sequencing

16S rRNA gene libraries were constructed using primers designed to amplify the V4 region (Kozich, Westcott, Baxter, Highlander, &
was the manufacturer’s instructions. The sequencing chemistry utilized placed on ice before loading into Illumina MiSeq cartridge following for each paired-end read using via Quince, & Knight, 2011). OTU taxonomy assignment was performed selection utilized USEARCH (Edgar, 2010; Edgar, Haas, Clemente, UCHIME reference “Gold” database. This step along with the OTU were done via the QIIME script using the v.1.7) pipeline (Caporaso et al., 2010). Chimera checking and removal was msfseq_reagent_kit v2 (500 cycles) with run metrics of 250 cycles for each paired-end read using msfseq_control Software 2.2.0 and RTA 1.17.28.

2.6 | Analyses of sequenced samples

Overlapping 250-bp paired-end MiSeq amplicon reads were quality-filtered and merged via the illumina-utils software (Eren et al., 2013) to generate high-quality sequences spanning the V4 region. Quality filtering was only carried out on mismatches in the overlapping region between read pairs using a minimum overlap (−min-overlap-size) of 200 nt, a minimum quality Phred score (−min-qual-score) of Q20 and allowing a maximum of 5 mismatches per 100 nt (−p 0.05) over the 200-nt overlapping region.

Read pairs passing the filtering criteria were merged and analysed using the Quantitative Insights Into Microbial Ecology (QIIME v.1.7) pipeline (Caporaso et al., 2010). Chimera checking and removal were done via the QIIME script identify_chimeric_seqs.py using the UCHIME reference “Gold” database. This step along with the OTU selection utilized USEARCH (Edgar, 2010; Edgar, Haas, Clemente, Quince, & Knight, 2011). OTU taxonomy assignment was performed via QIIME’s pick_open_reference_otus.py function using the 13.8 version of the Greengenes database (McDonald et al., 2012), a 97% similarity threshold for OTU formation and a minimum cluster size of 2 (i.e., each OTU must contain at least two sequences). Technical replicates were collapsed, low abundance OTUs (<0.01% total) removed via filter_otus_from_otu_table.py (−min_count_fraction = 0.001) and samples rarefied to an even depth equivalent to the number of sequences present in the sample with the fewest number of reads (14,683 reads in total). The raw sequences are available online at the European Nucleotide Archive under Accession number ENA: PRJEB28621.

2.7 | Data analyses

Statistical analyses of community composition were performed in R (version 3.1.2; R Core Team, 2013) using the vegan (Oksanen, Kindt, & Legendre, 2007) and phyloseq (McMurdie & Holmes, 2013) packages. Following calculation of rarefaction curves in MacQIIME, a range of alpha diversity metrics were calculated: Simpson index (Simpson, 1949), OTU counts, Pielou evenness (Pielou, 1966) and phylogenetic diversity (Faith, 1992) were determined. Between-community diversity was calculated using Bray–Curtis dissimilarity (Bray & Curtis, 1957), Jaccard Index (Jaccard, 1912) and UniFrac (weighted and unweighted), a phylogeny-based dissimilarity matrix (Lozupone & Knight, 2005). The homogeneity of sample group dispersions (i.e., comparison of the magnitude of within-community diversity) was determined using the vegan function betadisper and significance assessed using permutation tests (PERMDISP). Statistical significance of the sample groupings (i.e., ancestral–descendant communities) was determined via permutilation multivariate analysis of variance (PERMANOVA) implemented in vegan as the adonis function (Oksanen et al., 2007). Mantel tests (Mantel, 1967) were used to assess the influence of community dissimilarity on difference in biogas production. For composition analysis, sequencing data were prefiltered to include only OTUs present at a frequency of more than 0.1% of total reads to avoid interferences from very rare OTUs, which may be errors. To determine whether there were any groups of organisms abundant in only one type of samples, communities were analysed at the phylum level using group_significance.py in MacQIIME. LefSE (Segata et al., 2011) was then used to determine differences in the frequency at the genus level between ancestral–descendant samples, as well as endpoint samples from good–bad gas producers (cumulative production of respectively more, or less than 3,000-mL gas in the experiment). We also looked at the abundances of the archaean reads, looking at the differences between the two types of methanogens: acetoclastic, that use acetate to produce methane, and hydrogenotrophic, that produce methane using carbon dioxide and hydrogen as substrate.

To determine how community composition affected gas production, cumulative gas production was independently regressed against diversity and density metrics. To determine functional saturation, the natural logarithm of cumulative gas production was regressed against the natural logarithm of species richness (Reich, Tilman, & Isbell, 2012). The value of the exponent (b) of this function is an indicator of the functional saturation.

We determined whether between-community diversity was significantly different than null communities randomly generated from the data sets, after controlling for within-community diversity (Chase, 2010). For the purpose of this comparison, we generated 1,000 null communities and used their mean as a community formed by pure stochastic process. We compared it with real-life data, testing the null hypothesis that there is no difference between expected and observed between-community diversity using the “oecosimu” function in vegan package for R (Oksanen et al., 2007).

In order to assess which factors are likely to be the main direct and indirect drivers of gas production in our correlational study, we applied path analysis (Grace et al., 2012; Yvon-Durocher et al., 2015), where we use structural model equations using variables that had significant relationships with gas production (species richness, and archaean and bacterial densities). We employed simplifying multiple hierarchical linear mixed effects models based on all combinations of plausible hypotheses (17, in total) about how...
the variables affect each other and gas production. Models were fitted using lme function in nlme package in R. We calculated the Akaike information criterion (AIC) scores of the models that were statistically significant using “lavaan” package for R (Rosseel, 2012) and used them to pick the model that best fitted the data. We compared the importance of particular paths in the final model using standardized coefficients that indicate a percentage change in gas production.

3 | RESULTS

3.1 | Natural communities

3.1.1 | Compositional changes through time

Community composition converged after eight weeks of cultivation as shown by the decrease in between-community (beta) diversity of postexperiment communities comparing to pre-experiment communities (Figure 1a; Permdisper, $F_{1,22} = 12.38; p = 0.002$). There was also a moderate but significant separation between descendant and ancestral communities (Figure 1a; Bray–Curtis distance; PERMANOVA: $R^2 = 0.19, p < 0.001$). Note that this convergence was also robust to different distance measures: unweighted and weighted UniFrac and Jaccard index ($p < 0.01$ in all cases). Net alpha diversity (OTU read counts and reciprocal Simpson's index) decreased between ancestral and descendant communities ($F_{1,11} = 6.24, p = 0.03; F_{1,11} = 6.97, p = 0.02$; Figure 1b), but community convergence was not simply the result of this loss of diversity, shown with a permutation test comparing observed beta diversity of descendant communities against null communities ($p < 0.01$). The major change in community composition through time with respect to specific taxa was an increase in the frequency of Firmicutes reads (37.9%–68.0%), a decrease in the frequency of Bacteriodetes reads (30.1%–7.75%) and a decrease in the frequency of Proteobacteria reads (9.85%–0.86%) (Figure 1c).

In general, we observed large changes in composition through time. A high proportion of OTUs were lost through time (between 34 and 72%). Similarly, a large proportion (between 17 and 65%) of OTUs that were present in the descendant samples were below detection levels in the ancestral samples. These changes had a significant impact on the community composition as shown by the difference between the pre- and postexperiment beta diversity when looking when using community distance is based on the presence/absence of taxa rather than relative frequency.

3.1.2 | Linking community composition with biogas production

We investigated how the characteristics of communities pre- and postexperiment affect total gas production. We used the cumulative gas production (averaged across replicates) value as the proxy for community performance for the tests, as there was little variability in terms of gas production ranks between weeks. For example, Spearman rank correlation coefficients between total, week one and week eight gas production ranged from 0.75 to 0.93, $p < 0.01$ in all cases. Cumulative gas production was not affected by feed type ($F_{1,33} = 1.33, p = 0.3$).

For starting communities, we found no significant correlations between gas production and either archaeal copy number, alpha diversity metrics ($p > 0.2$ in all cases) or pairwise beta diversity (Mantel test: $r = 0.2; p = 0.09$). Note that bacterial copy number was equalized at the start of the experiment. By contrast, after eight weeks of propagation, there was a positive relationship between archaeal
copy number and gas production (Figure 2a; \( F_{1,10} = 14.9, p = 0.003 \); 83% of archaeal amplicon sequence reads were methanogens). There was no additional effect of bacterial copy number (\( F_{1,9} = 0.2, p > 0.2 \)). There were also no relationships between biogas production and alpha diversity metrics except for a positive relationship between gas production and species (OTU) richness (Figure 2b; \( F_{1,10} = 12.5, p < 0.005 \)). Unsurprisingly, communities that showed the greatest relative loss of OTUs through time produced the least gas (Spearman \( R = -0.67, p < 0.05 \)). The slope of the natural logarithms of gas production and OTU number was 4.07, suggesting an accelerating effect of increasing OTUs on methane production. Finally, pairwise beta diversity correlated with differences in gas production (Mantel \( r = 0.54, p = 0.001 \); the greater the difference in gas production, the bigger the difference in community composition.

We used path analysis to infer the likely causal relationships between gas production, species richness, and bacterial and archaeal densities. Comparisons of AIC scores (Table S3) of 17 hypothetical paths suggest that the interaction of bacterial biomass and species richness drives archaeal abundance, which leads to higher gas production (Figure S1).

### 3.1.3 Linking biogas production to specific taxa

We also investigated how the frequencies of specific taxa might be associated with gas production (Table S2). Of particular note, there was a positive correlation between gas production and the proportion of *Methanosarcina*, a genus of acetoclastic methanogenic Archaea (\( F_{1,10} = 3.9, p < 0.001 \); Figure 3).

### 3.2 Diluted communities

#### 3.2.1 Linking community composition with biogas production

To experimentally manipulate diversity, we diluted a mixed community over six orders of magnitude followed by a period of regrowth to allow equal numbers of cells to be inoculated across treatments. The manipulation worked: There were fewer OTUs detectable with increasing dilution (\( F_{3,35} = 21.8; p < 0.001 \)). Crucially, we found a positive relationship between biogas production and number of

#### 3.2.2 Linking biogas production to specific taxa

Three OTUs belonging to family Coriobacteriaceae, Ruminococcaceae and Peptococcaceae were significantly overrepresented in the less diluted samples (Kruskal–Wallis test, Bonferroni \( p < 0.05 \)).
We investigated the link between microbial diversity and the rate of methane production in natural and manipulated communities. We found no correlations between any aspect of community composition at the start of the experiment and methane production across the 12 natural communities. However, after eight weeks of propagation in laboratory anaerobic digesters, there was a loss of diversity within communities and communities had converged. At this point, we found a positive relationship between methane production, species (OTU) richness, bacteria and methanogen density. We obtained the same qualitative results in communities where diversity was manipulated by dilution over six orders of magnitude. This suggests that decreasing species richness in methanogenic communities will reduce methane production and that this effect is robust to variation in species composition present in natural communities.

Manipulating diversity by dilution has limitations. Most obviously, it confounds diversity with species identity to some extent, in that dilution of communities results in the loss of predominantly rare taxa. As a consequence, the results suggest that methane production decreases with the increasing loss of rare species, rather than the loss of random taxa. To put this into context, the loss of half
of the community made up by the rarest species results in approximately 50% reduction in gas production. Dilution also had the effect of increasing within-treatment beta diversity, which could limit the interpretation of analyses. This increase in beta diversity is presumably the result of increased stochasticity in community assembly when taxa are at lower frequencies as a result of dilution.

The relationship between gas production and species richness in the dilution experiment showed little functional saturation (an exponent of 0.43 for the relationship) compared to most diversity-function studies (O’Connor et al., 2017). By contrast, the exponent of the gas production-species richness relationship in the correlative study was extremely high (~4), suggesting an accelerating relationship. However, this very high value likely reflects an overestimation of species richness of the poorer-performing communities. Specifically, poor-performing communities had the greatest net loss of OTUs through time, and this loss may be underestimated because of residual DNA of dead cells and the presence of OTUs that were not yet driven to extinction. Our study supports the growing body of evidence that rare species play an important role in the community function (Lynch & Neufeld, 2015; Mouillot et al., 2013).

Both our studies that suggest large numbers of rarer species support higher densities of aceticlastic methanogens: methane-producing Archaea locked into mutualisms with acetate-producing bacteria (Ferry, 2012), which are locked into syntrophic cross-feeding interactions with acetate-producing bacteria. Precisely why this might be is unclear, but recent theory suggests that growth under low energy conditions (as is the case under anaerobic conditions when oxygen is not used as the final electron receptor) is typically thermodynamically constrained, and results in a high diversity of metabolic niche specialists. This is because there a selective advantage to use a substrate in different way to competitors (negative frequency-dependent selection (Clarke, 1979), to avoid thermodynamic inhibition of metabolism resulting from the build up of waste products (Großkopf & Soyer, 2016). More generally, thermodynamic constraints may help to explain why diversity seems less important for aerobic (Nielsen et al., 2011) than anaerobic (e.g., methanogenesis and denitrification; Phillipot et al., 2013) functions in communities approaching natural levels of diversity. Finally, it is also possible that genetic variation within species, which would have been reduced by dilution and perhaps during propagation of the natural communities, could have contributed to the results. For example, recent work suggests that within-species diversity associated with rapid adaptation can play a major role in the structure of natural soil microbial communities (Gómez et al., 2016).

The composition of the communities we investigated was broadly typical of methanogenic communities (Nelson, Morrison, & Yu, 2011; Yang et al., 2014; Yu, Lee, & Hwang, 2005), with Firmicutes, Bacteroides and Proteobacteria being the main phyla. However, consistent with other studies (De Vrieze et al., 2015; Demirel & Yenigün, 2006; Elsheshbisy, Naklha, & Hafez, 2012; Mladenovska et al., 2003; Regueiro et al., 2012; Town, Links, Fonstad, & Dumonceaux, 2014; Vanwanterghem et al., 2014), we observed a convergence of communities through time. This was associated with an increase in Firmicutes and a decline in Bacteriodetes reads through time in the 12 natural communities. The most predominant group in the Firmicutes, Clostridia, is known for their cellulolytic and amyloytic activity (Nelson et al., 2011). Our medium was based on starch and cellulose, making Clostridia perfect candidates for the hydrolysis steps of fermentation within the system. Another reason for the increase in Firmicutes could simply be selection against them during sampling: Firmicutes have low oxygen tolerance (Kampmann et al., 2012), and while every care was taken during sampling, initial communities were inevitably exposed to air in the field. It is less clear why Bacteriodetes were selected against in the laboratory-scale anaerobic digesters, but their reduction in frequency is consistent with increased biogas production: Bacteriodetes are associated with the production of propionate and other short fatty acids, which can lead to disturbances in anaerobic digester system (Gallert & Winter, 2008).

It was difficult to draw any firm conclusions about the role of specific taxa in gas production, beyond the positive effect of aceticlastic methanogens. However, in the natural converged communities, poor gas production was associated with the presence of Pseudorabibacter, Oscillospira, Bacteroides uniformis and Enterobacteriaceae. These species are typically associated with animal gut microbiomes, where they putatively are responsible for fermentation of glycan to butyrate (Benítez-Páez, Gómez del Pulgar, & Sanz, 2017). It is possible that our medium, rich in meat extract, contributed to the enrichment of these species. The lack of animal host able to metabolize butyrate may have to its accumulation, detrimental to the functioning of the communities not capable of coping with it. OTUs that were overrepresented in the more diverse communities in the dilution experiment could plausibly have important roles: Coriobacteriaceae have been suggested before to play a role in breaking down aromatic compounds in (Noguchi, Kurisu, Kasuga, & Furumai, 2014); Ruminococcus are involved in cellulolytic and xylolytic activity (Jia, Wilkins, Lu, Cai, & Lee, 2016); and Peptococcus are speculated to be acetate-producing syntrophic partners of aceticlastic methanogens (Tang, Shigematsu, Morimura, & Kida, 2005).

The importance of rare species in determining the productivity of methanogenic communities has potentially important implications. First, communities may take a relatively long time to achieve maximal levels of methane production following environmental changes, given that key beneficial rare species may not be present. This is in contrast to aerobic communities where function is typically restored to high levels following environmental change because of functional redundancy within communities (Martiny et al., 2006; Strickland, Lauber, Fierer, & Bradford, 2009). Second, from a biotechnological perspective, we demonstrate, like research before us, that the starting inoculum plays a crucial role (De Vrieze et al., 2015; Elsheshbisy et al., 2012). Unfortunately, our results show that knowledge of the starting inoculum a priori may prove uninformative as the importance of community composition only becomes apparent after ecological selection imposed by the specific anaerobic digester conditions. This problem can be circumvented by inoculating multiple communities in the starting...
culture (Sierocinski et al., 2017). In summary, our results suggest that there is little functional redundancy in methanogenic communities, and hence, any loss of diversity will likely reduce methane production. Moreover, given that microbes appear to be dispersal-limited to some extent (Bell, 2010), the potential for methanogenic communities to adapt to changing conditions is likely to be constrained by their starting composition.

ACKNOWLEDGEMENTS

We would like to thank the companies that kindly provided us with the samples for the experiments. The work was funded by the BBSRC (UK), NERC and the AXA Research Fund. AB is also funded by the Royal Society (UK).

DATA ACCESSIBILITY

Sequencing data from these experiments will be stored in a publicly accessible repository. The raw sequences are available online at the European Nucleotide Archive under Accession number ENA: PRJEB28621.

AUTHOR CONTRIBUTIONS

P.S., A.B., F.B., O.S.S., T.G. and P.J.H. conceived the experiments. P.S. and F.B. collected the data and conducted the experiments. M.B. and F.B. were responsible for sequencing and sequence data preparation. A.B., P.S. and G.Y.D. did the statistical analyses. A.B. and P.S. wrote the manuscript. All authors contributed to the revisions.

ORCID

Pawel Sierocinski https://orcid.org/0000-0001-9034-5624

REFERENCES

Bell, T. (2010). Experimental tests of the bacterial distance-decay relationship. ISME Journal, 4(11), 1357–1365. https://doi.org/10.1038/ismej.2010.77

Bell, T., Newman, J. J. A., Silverman, B. B. W., Turner, S. L. S., & Lilley, A. A. K. (2005). The contribution of species richness and composition to bacterial services. Nature, 436(7054), 1157–1160. https://doi.org/10.1038/nature03891

Benítez-Páez, A., Gómez del Pulgar, E. M., & Sanz, Y. (2017). The glycolytic versatility of bacteroides uniformis CECT 7771 and its genome response to oligo and polysaccharides. Frontiers in Cellular and Infection Microbiology, 7, 383. https://doi.org/10.3389/fcimb.2017.00383

Bray, J., & Curtis, J. (1957). An ordination of the upland forest communities of southern Wisconsin. Ecological Monographs, 27(4), 325–349. https://doi.org/10.2307/1942268

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. Nature Methods, 7(5), 335–336. https://doi.org/10.1038/nmeth.f.303

Chase, J. (2010). Stochastic community assembly causes higher biodiversity in more productive environments. Science, 328(5984), 1388–1391. https://doi.org/10.1126/science.1187820

Clarke, B. C. (1979). The evolution of genetic diversity. Proceedings of the Royal Society B: Biological Sciences, 205(1161), 453–474. https://doi.org/10.1098/rspb.1979.0079

De Vrieze, J., Raport, L., Willems, B., Verbruggen, S., Volcke, E., Meers, E., & Boon, N. (2015). Inoculum selection influences the biochemical methane potential of agro-industrial substrates. Microbial Biotechnology, 8(5), 776–786. https://doi.org/10.1111/1751-7915.12268

Delgado-Baquerizo, M., Maestre, F. T., Reich, P. B., Jeffries, T. C., Gaitan, J. J., Encinar, D., & Singh, B. K. (2016). Microbial diversity drives multifunctionality in terrestrial ecosystems. Nature Communications, 7(JANUARY), 1–8. https://doi.org/10.1038/ncomms10541

Demirel, B., & Yenigün, O. (2006). Changes in microbial ecology in an anaerobic reactor. Bioresource Technology, 97(10), 1201–1208. https://doi.org/10.1016/j.biortech.2005.05.009

Dolezel, J., Bartos, J., Voglmayr, H., & Greilhuber, J. (2003). Nuclear DNA content and genome size of trout and human. Cytometry. Part A: The Journal of the International Society for Analytical Cytology, 51(2), 127–128. https://doi.org/10.1002/cyt.a.10013 https://doi.org/10.1002/jissn1097-0320

Edgar, R. R. C. (2010). Search and clustering orders of magnitude faster than BLAST. Bioinformatics, 26(19), 2460–2461. https://doi.org/10.1093/bioinformatics/btq461

Edgar, R. C., Haas, B. J., Clemente, J. C., Quince, C., & Knight, R. (2011). UCHIME improves sensitivity and speed of chimera detection. Bioinformatics, 27(16), 2194–2200. https://doi.org/10.1093/bioinformatics/btr381

Elbeshbishy, E., Nakhla, G., & Hafez, H. (2012). Biochemical methane potential (BMP) of food waste and primary sludge: Influence of inoculum pre-incubation and inoculum source. Bioresource Technology, 110, 18–25. https://doi.org/10.1016/j.biortech.2012.01.025

Eren, A. M., Maignien, L., Sul, W. J., Murphy, L. G., Grim, S. L., Morrison, H. G., & Sogin, M. L. (2013). Oligotyping: Differentiating between closely related microbial taxa using 16S rRNA gene data. ISME Journal, 7(4), 27–37. https://doi.org/10.1038/ismej.2012.101

Faith, D. (1992). Conservation evaluation and phylogenetic diversity. Biological Conservation, 61(1), 1–10. https://doi.org/10.1016/0006-3207(92)91201-3

Ferry, J. (2012). Methanogenesis: Ecology, physiology, biochemistry & genetics. Berlin: Springer Science & Business Media.

Fierer, N., Leff, J. W., Adams, B. J., Nielsen, U. N., Bates, S. T., Lauber, C. L., & Caporaso, J. G. (2012). Cross-biome metagenomic analyses of soil microbial communities and their functional attributes. Proceedings of the National Academy of Sciences of the United States of America, 109(52), 21390–21395. https://doi.org/10.1073/pnas.1215210110

Gallert, C., & Winter, J. (2008). Propionic acid accumulation and degredation during restart of a full-scale anaerobic biowaste digester. Bioresource Technology, 99(1), 170–178. https://doi.org/10.1016/j.biortech.2006.11.014

Gómez, P., Paterson, S., De Meester, L., Liu, X., Lenzi, L., Sharma, M. D., & Buckling, A. (2016). Local adaptation of a bacterium is as important as its presence in structuring a natural microbial community. Nature Communications, 7, 12453. https://doi.org/10.1038/ncomms12453

Grace, J., Schoolmaster, D., Guntenspenger, G., Little, A., Mitchell, B., Miller, K., & Schweiger, E. (2012). Guidelines for a graph-theoretic implementation of structural equation modeling. Ecosphere, 3(8), 1–44. https://doi.org/10.1890/ES12-00048.1
Salonius, P. O. (1981). Metabolic capabilities of forest soil microbial populations with reduced species diversity. *Soil Biology and Biochemistry*, 13(1965), 1–10. https://doi.org/10.1016/0038-0717(81)90094-8

Segata, N., Izard, J., Waldron, L., Gevers, D., Miropolsky, L., Garrett, W. S., & Huttenhower, C. (2011). Metagenomic biomarker discovery and explanation. *Genome Biology*, 12(6), R60. https://doi.org/10.1186/gb-2011-12-6-r60

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 communities. *Current Biology*, 27(21), 3390–3395. https://doi.org/10.1016/j.cub.2017.09.056

Simpson, E. (1949). Measurement of diversity. *Nature*, 163, 688. https://doi.org/10.1038/163688a0

Strickland, M. S., Lauber, C., Fierer, N., & Bradford, M. A. (2009). Testing the functional significance of microbial community composition. *Ecology*, 90(2), 441–451. https://doi.org/10.1890/08-0296.1

Tilman, D. (1997). Biodiversity and ecosystem functioning. In G. C. Daily (Ed.), *Nature’s services: Societal dependence on natural ecosystems* (pp. 93–112). Washington, D.C.: Island Press.

Town, J. R., Links, M. G., Fonstad, T. A., & Dumonceaux, T. J. (2014). Molecular characterization of anaerobic digester microbial communities identifies microorganisms that correlate to reactor performance. *Bioresource Technology*, 151, 249–257. https://doi.org/10.1016/j.biortech.2013.10.070

Vanwongtherem, I., Jensen, P., Dennis, P., Hugenholz, P., Rabaey, K., & Tyson, G. (2014). Deterministic processes guide long-term synchronised population dynamics in replicate anaerobic digesters. *The ISME Journal*, 8, 2015–2028. https://doi.org/10.1038/ismej.2014.50

Venkiteshwaran, K., Milferstedt, K., Hamelin, J., Fujimoto, M., Johnson, M., & Zitomer, D. H. (2017). Correlating methane production to microbiota in anaerobic digesters fed synthetic wastewater. *Water Research*, 110, 161–169. https://doi.org/10.1016/j.watres.2016.12.010

Wagg, C., Bender, S. F., Widmer, F., & van der Heijden, M. G. A. (2014). Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences of the United States of America*, 111(14), 5266–5270. https://doi.org/10.1073/pnas.1320054111

Wagner, R., Zona, D., Oechel, W., & Lipson, D. (2017). Microbial community structure and soil pH correspond to methane production in Arctic Alaska soils. *Environmental Microbiology*, 19(8), 3398–3410. https://doi.org/10.1111/1462-2920.13854

Yang, Y., Yu, K., Xia, Y., Lau, F. T. K., Tang, D. T. W., & Fung, W. C. (2014). Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants. *Applied Microbiology and Biotechnology*, 98, 5709–5718. https://doi.org/10.1007/s00253-014-6548-0

Yavitt, J. B., Yashiro, E., Cadillo-Quiroz, H., & Zinder, S. H. (2012). Methanogen diversity and community composition in peatlands of the central to northern Appalachian Mountain region, North America. *Biogeochemistry*, 109(1–3), 117–131. https://doi.org/10.1007/s10533-011-9644-5

Yu, Y., Lee, C., & Hwang, S. (2005). Analysis of community structures in anaerobic processes using a quantitative real-time PCR method. *Water Science & Technology*, 52, 85–91. https://doi.org/10.2166/wst.2005.0502

Yvon-Durocher, G., Allen, A. P. A., Cellamare, M., Dossena, M., Gaston, K. J., Leitao, M., & Trimmer, M. (2015). Five years of experimental warming increases the biodiversity and productivity of phytoplankton. *PLoS Biology*, 13(12), e1002324. https://doi.org/10.1371/journal.pbio.1002324

**Supporting Information**

Additional supporting information may be found online in the Supporting Information section at the end of the article.

**How to cite this article:** Sierocinski P, Bayer F, Yvon-Durocher G, et al. Biodiversity–function relationships in methanogenic communities. *Mol Ecol*. 2018;27:4641–4651. [https://doi.org/10.1111/mec.14895](https://doi.org/10.1111/mec.14895)