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Supplementary Information

Microbial Community Composition and Diversity in Rice Straw Co-digestion Bioreactors with Dairy Manure

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Additional Methods in Next Generation Sequencing:

The PCRs included 1 to 10 ng of DNA extract (total volume 1μl), 15 pmol of each forward primer and reverse primer (in 20 μL volume of 1 x MyTaq buffer containing 1.5 units MyTaq DNA polymerase (Bioline) and 2 μl of BioStabII PCR Enhancer (Sigma). For each sample, the forward and reverse primers had the same 10-nt barcode sequence. PCRs were carried out for 30 cycles using the following parameters: 2 min 96°C pre-denaturation; 96°C for 15 s, 50°C for 30 s, 70°C for 90 s. DNA concentration of amplicons of interest was determined by gel electrophoresis. About 20 ng of amplicon DNA from each sample were pooled for up to 48 samples carrying different barcodes. If needed, PCRs with low yields were further amplified for 5 cycles.

The amplicon pools were purified with one volume AMPure XP beads (Agencourt) to remove primer dimer and other small mispriming products, followed by an additional purification on MinElute columns (Qiagen). About 100 ng of each purified amplicon pool DNA was used to construct Illumina libraries using the Ovation Rapid DR Multiplex System 1-96 (NuGEN). Illumina libraries were pooled and size selected by preparative gel electrophoresis. Sequencing was done on an Illumina MiSeq using V3 Chemistry (Illumina).

A total of 419,747 quality filtered samples were obtained from 15 samples with the number of 16S rDNA sequences ranging from 10,864 to 48,127 (mean, 27,983). After removing the chimera sequences the operational taxonomic units (OTUs) were picked at more than 97 % similarity representing 9,720 OTUs. The alpha and beta diversity of the microbial community were then assessed.

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