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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 1 µl), 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.

List of Figures:

- Figure S1:** Time-course data of digester performance including acclimation period (experiment 'Time 0' shown by dashed line) for pH, %Volatile Solids Removal (VSR), g VS/L, specific methane yields and VFA levels for: **a)** RS100, **b)** RS90, **c)** RS70, **d)** RS30.
- Figure S2:** Shared predominant OTU table to the genus level (only ≥ 0.5 % abundance) in bioreactor and raw dairy manure based on sample appearances. "H1", "H2", and "H3" refer to each HRT cycle (25 days) the reactors were operated.
- Figure S3:** Phylogenetic tree of predominant OTUs (only ≥ 0.5 % abundance) shared across all samples. Branch closeness and small white, grey, and black circles on branch junctions indicate percentage of similarity (50 - 69 %, 70 - 89 %, and 90+ %).
- Figure S4:** Extended error bar plot of significant differences between predominant OTUs in the RS100 reactor versus raw dairy manure (DM).
- Figure S5:** Extended error bar plot of significant differences between predominant OTUs in the RS90 reactor versus raw dairy manure (DM).
- Figure S6:** Extended error bar plot of significant differences between predominant OTUs in the RS70 reactor versus raw dairy manure (DM).
- Figure S7:** Extended error bar plot of significant differences between predominant OTUs in the RS30 reactor versus raw dairy manure (DM).

Figure S1

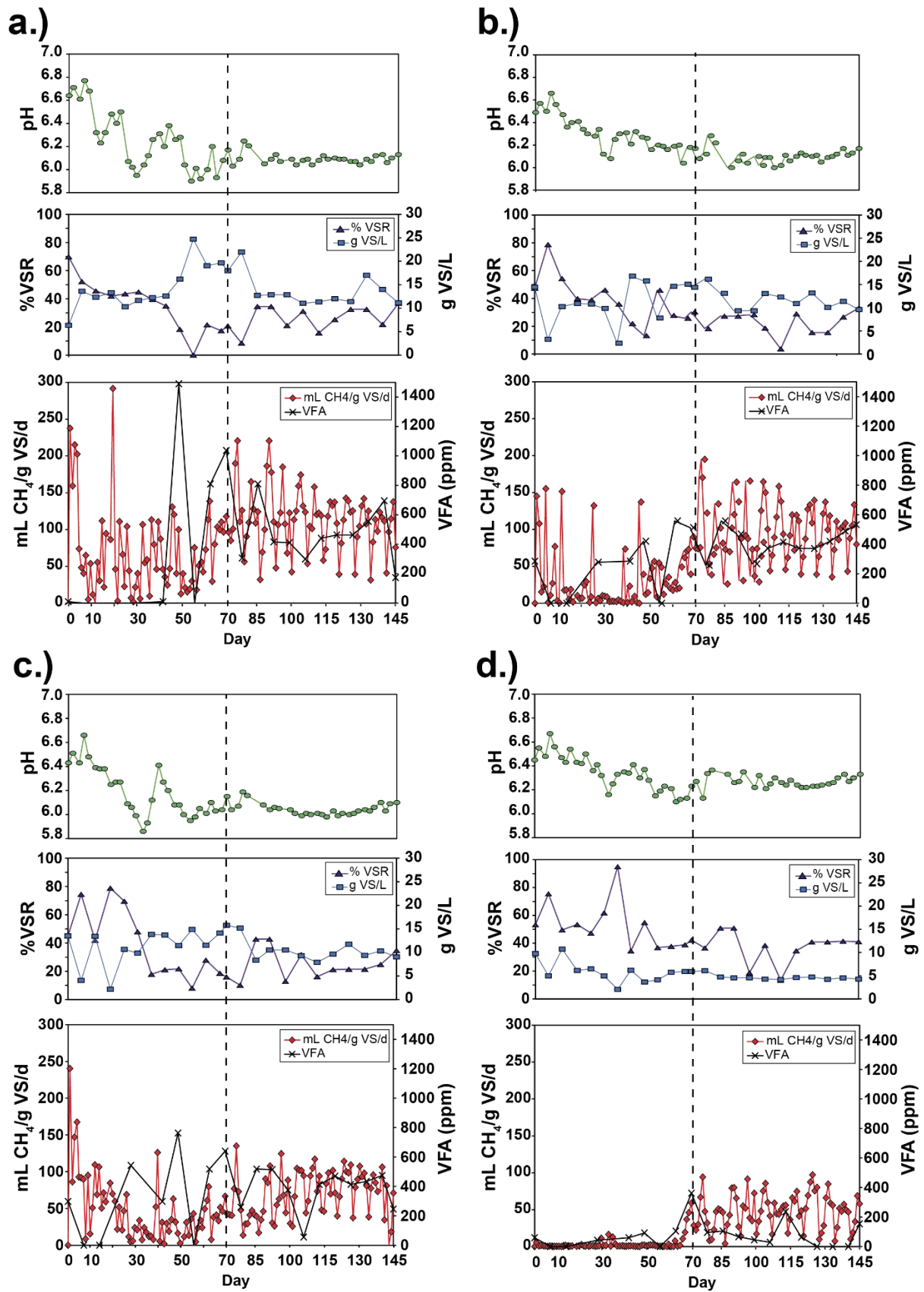
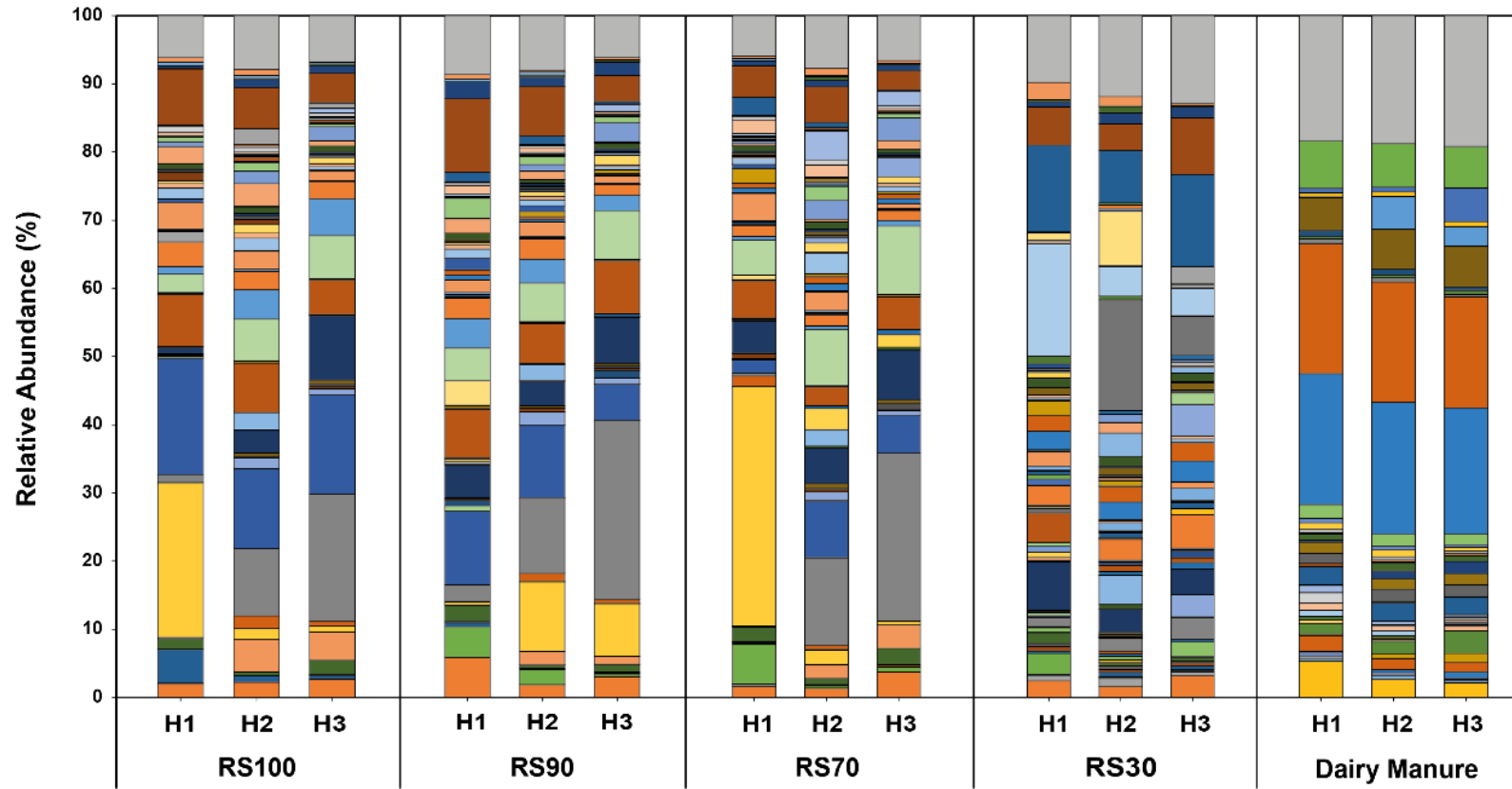


Figure S2



OTU 036	OTU 005	OTU 013	OTU 052	OTU 011	OTU 044	OTU 002	OTU 084	OTU 135	OTU 221	OTU 022
OTU 008	OTU 047	OTU 179	OTU 009	OTU 175	OTU 042	OTU 084	OTU 053	OTU 010	OTU 130	OTU 022
OTU 012	OTU 069	OTU 101	OTU 186	OTU 245	OTU 180	OTU 061	OTU 059	OTU 150	OTU 117	OTU 164
OTU 105	OTU 021	OTU 160	OTU 068	OTU 219	OTU 311	OTU 071	OTU 063	OTU 123	OTU 019	OTU 019
OTU 187	OTU 158	OTU 204	OTU 178	OTU 137	OTU 131	OTU 120	OTU 024	OTU 140	OTU 023	OTU 033
OTU 014	OTU 138	OTU 159	OTU 037	OTU 017	OTU 027	OTU 181	OTU 088	OTU 087	OTU 109	OTU 113
OTU 074	OTU 030	OTU 127	OTU 122	OTU 147	OTU 076	OTU 015	OTU 016	OTU 145	OTU 070	OTU 031
OTU 166	OTU 007	OTU 054	OTU 146	OTU 083	OTU 080	OTU 183	OTU 124	OTU 162	OTU 121	OTU 039
OTU 106	OTU 057	OTU 153	OTU 056	OTU 315	OTU 174	OTU 051	OTU 065	OTU 155	OTU 218	OTU 055
OTU 165	OTU 194	OTU 139	OTU 028	OTU 077	OTU 089	OTU 092	OTU 079	OTU 148	OTU 067	OTU 200
OTU 097	OTU 136	OTU 072	OTU 038	OTU 026	OTU 018	OTU 040	OTU 182	OTU 041	OTU 132	OTU 111
				OTU 062						
										Others (<0.5%)

Figure S3

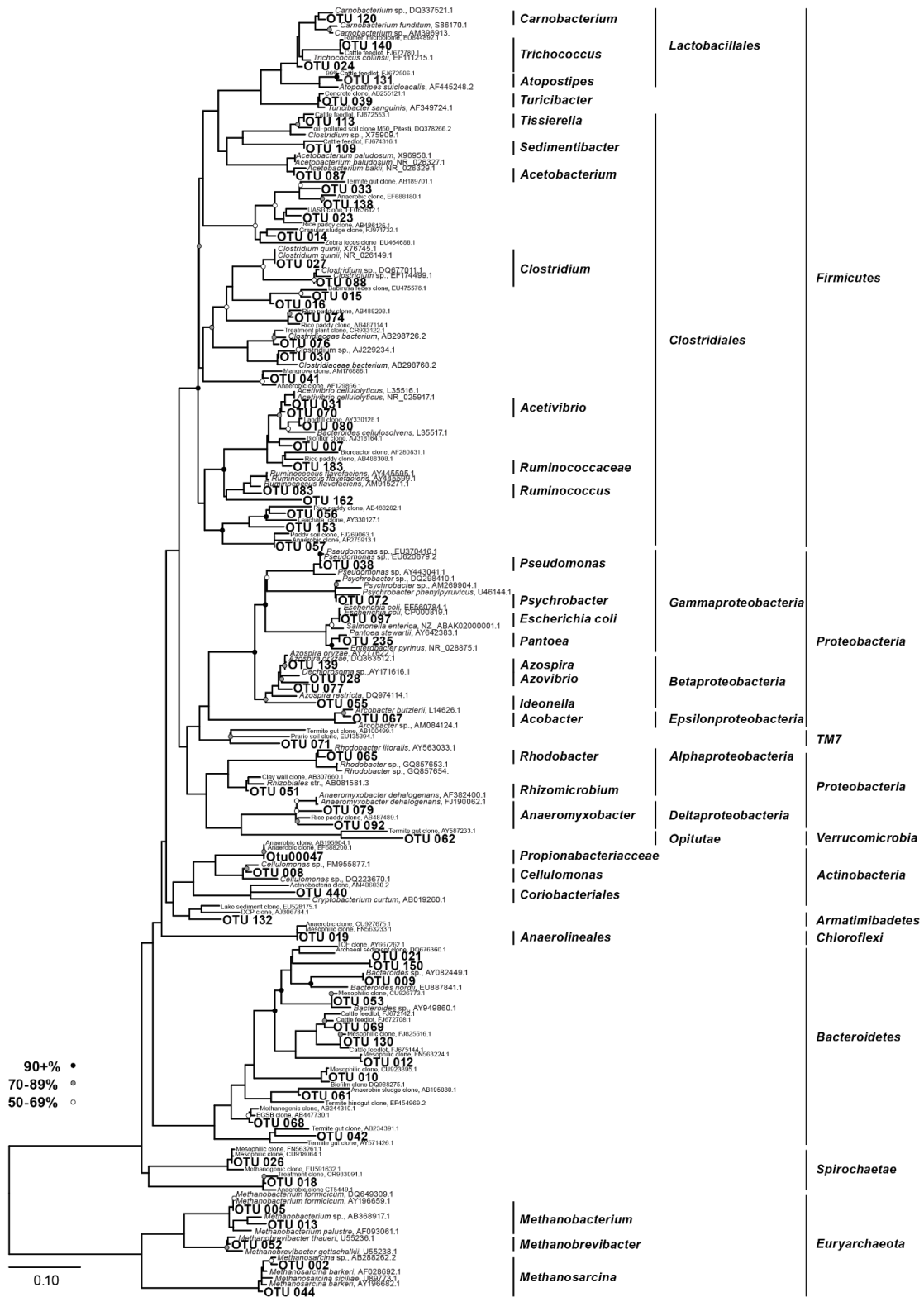


Figure S4

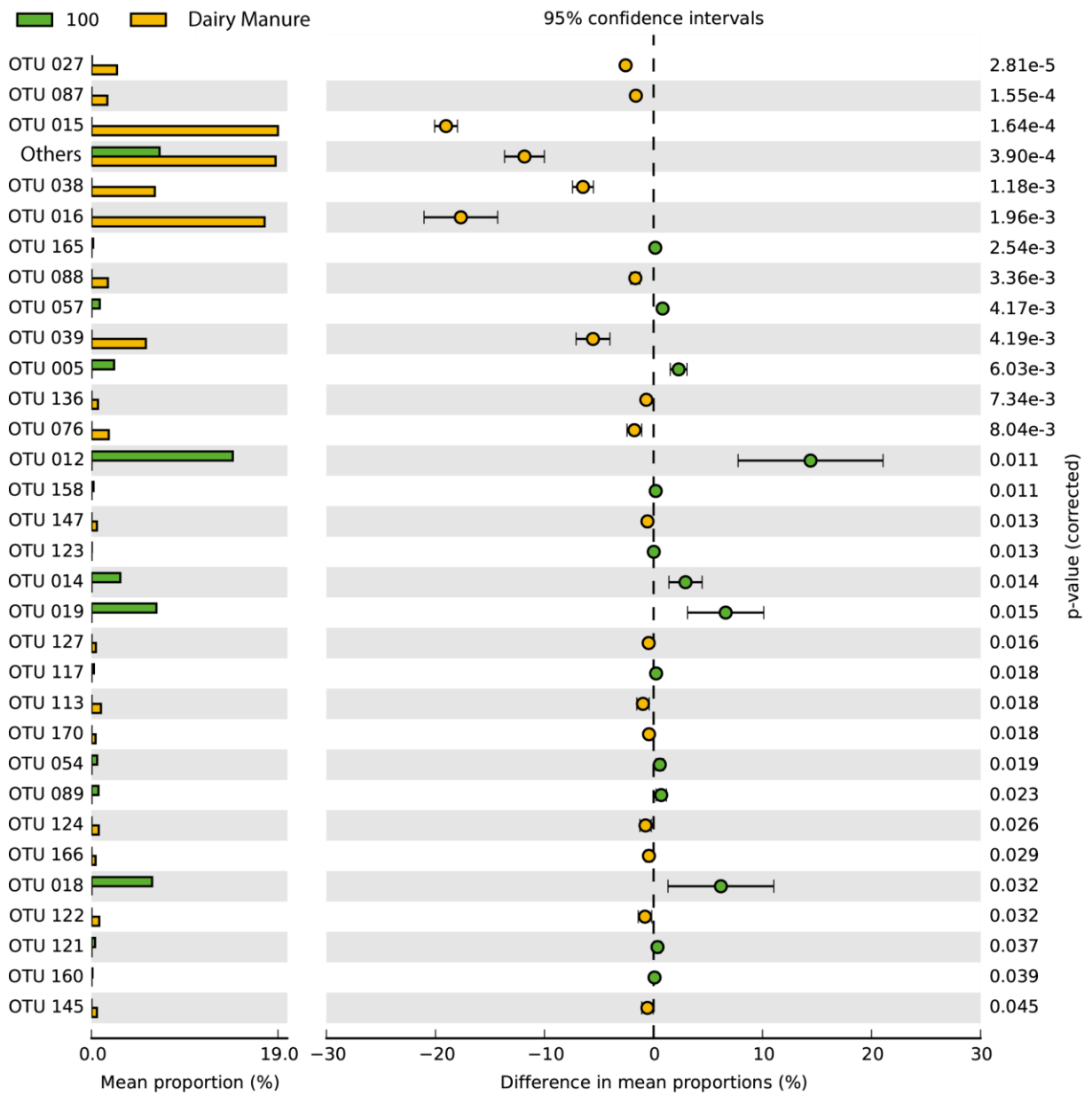


Figure S5

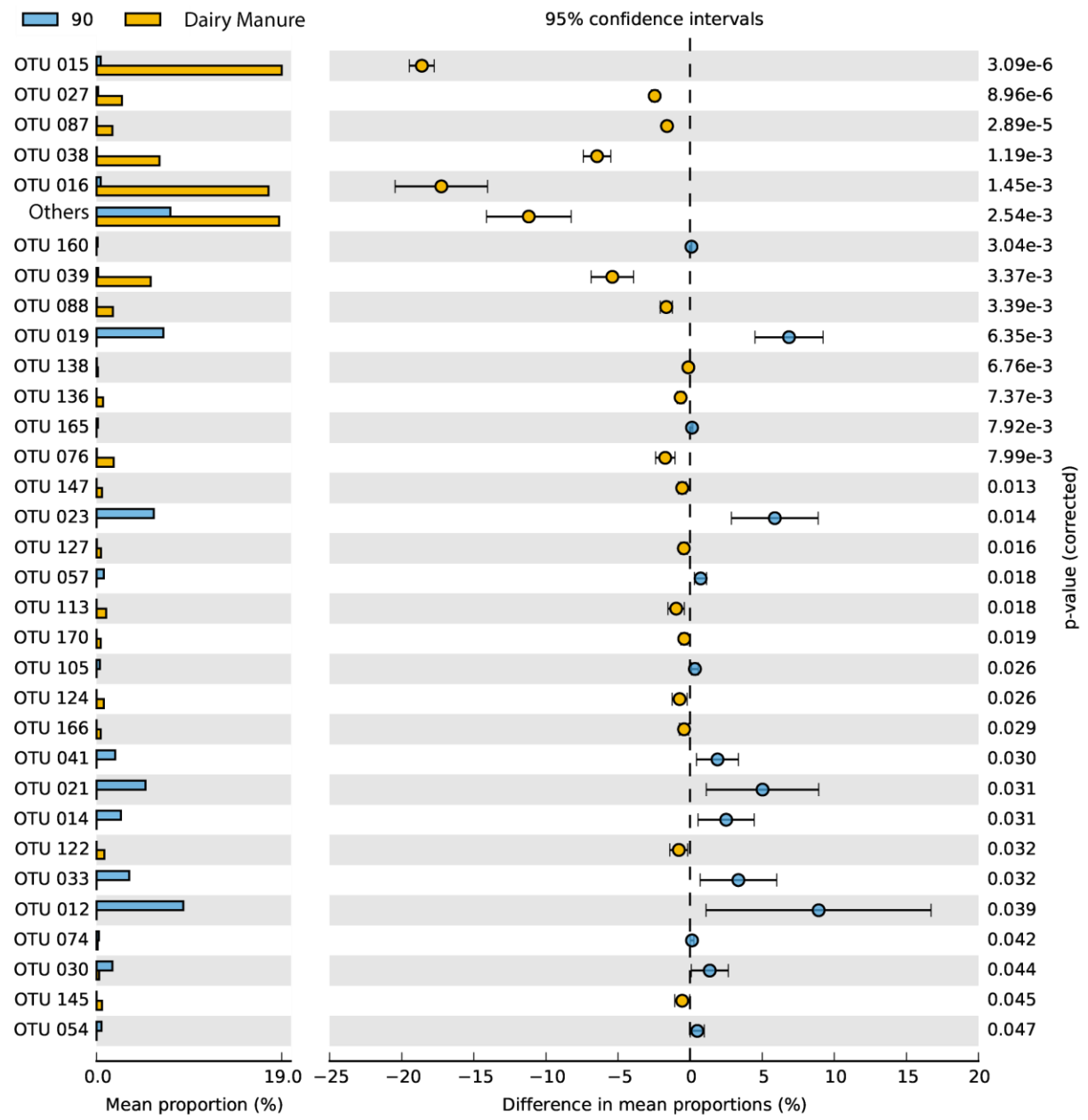


Figure S6

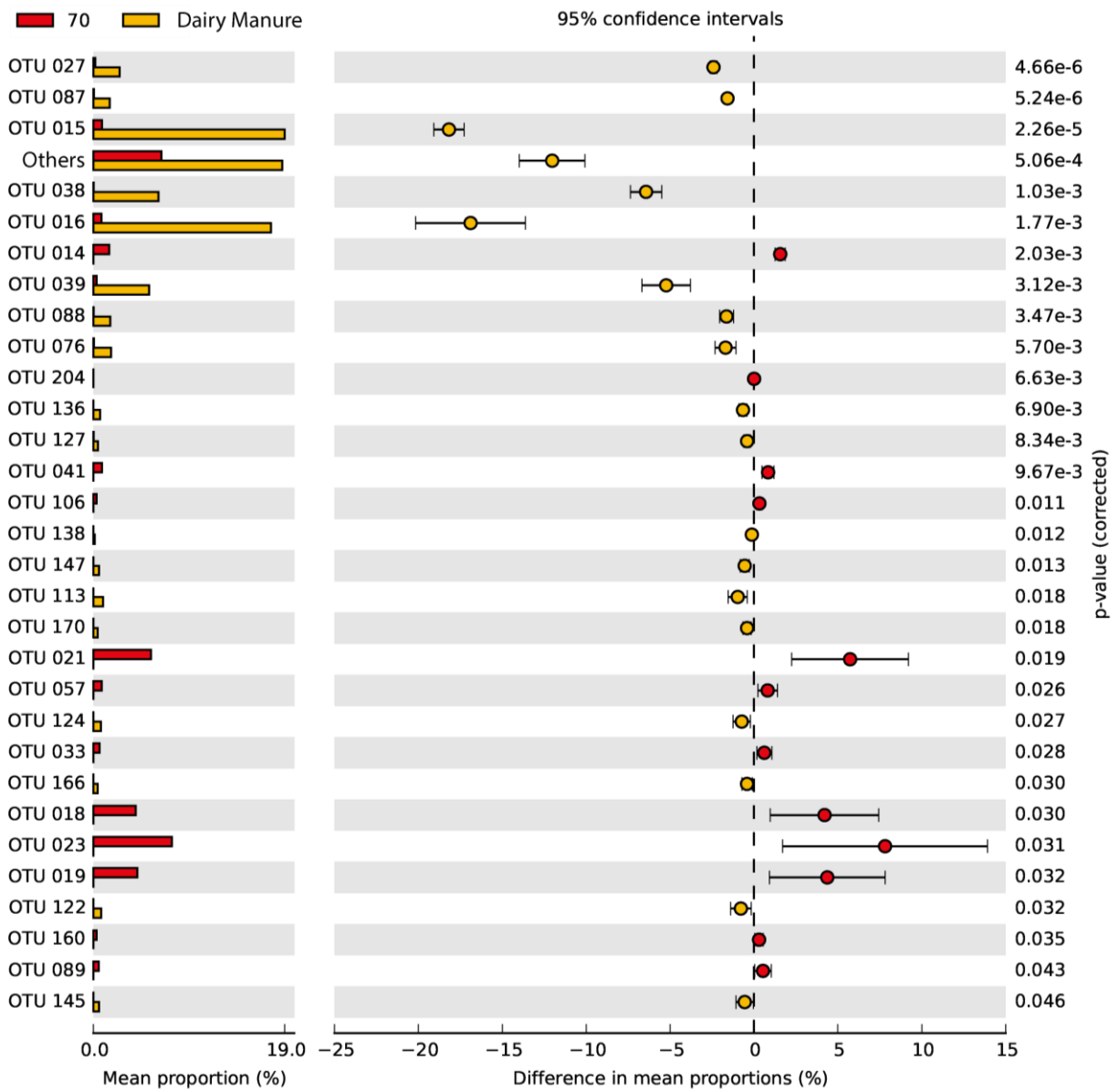


Figure S7

