

Supplementary Material: Decline in Performance of Biochemical Reactors for Sulphate Removal from Mine-Influenced Water is Accompanied by Changes in Organic Matter Characteristics and Microbial Population Composition

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Table S1. Sulphur balance over the BCRs and estimates of the masses of FeS formed within the BCRs over their period of operation.

Column	Amount of Sulphate Entering (mmoles)	Amount of Sulphate Leaving (mmoles)	Amount of Sulphate Removed (mmoles)	Amount of S Formed as Soluble Sulphide (mmoles)	Estimated FeS Formed (g)
BCR_W1	376	7.3	369	41	29.5
BCR_W2	932	78	854	250	54.4
BCR_W3	932	145	787	151	57.2
BCR_H1	408	50	358	28	29.7
BCR_H2	408	40	368	20	31.3
BCR_H3	956	82	874	125	67.4

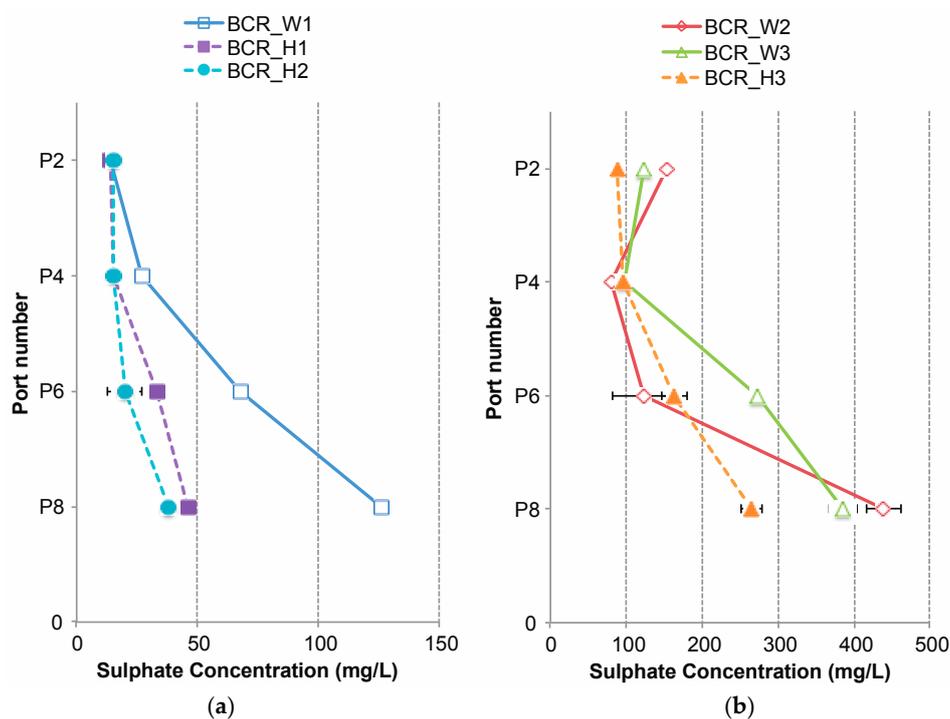
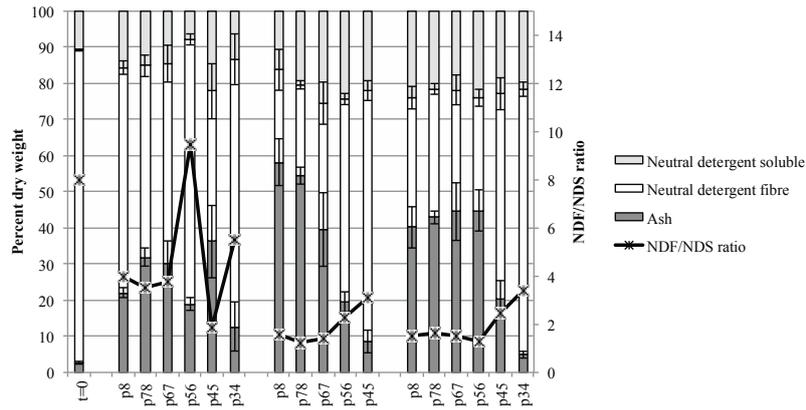
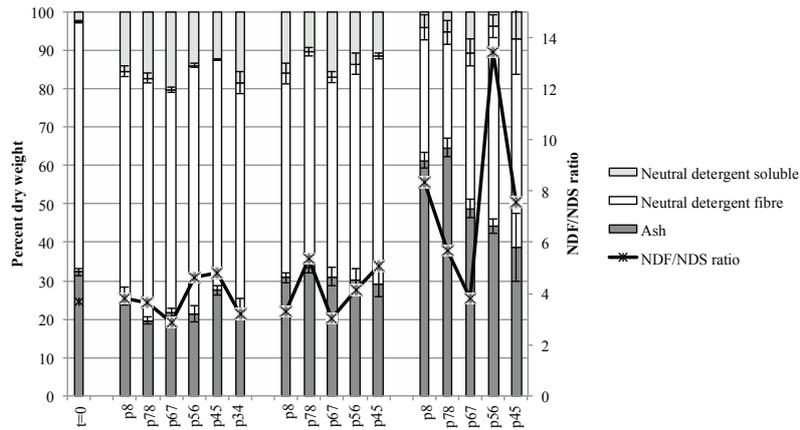


Figure S1. Sulphate concentrations measured in the pore water from Ports 8, 6, 4 and 2 for the (a) early; and (b) late sacrificed bioreactors. Port 8 was located 2cm above the influent and Port 2 was the effluent leaving the bioreactor matrix. BCR_W indicates a biochemical reactor containing the wood-rich mixture and BCR_H a biochemical reactor containing the hay-rich mixture.

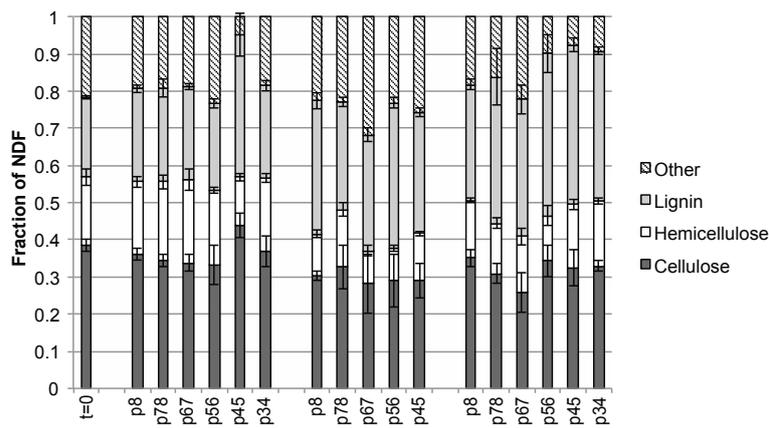


(a)



(b)

Figure S2. Composition of the solid matrices in the (a) wood-rich; and (b) hay-rich bioreactors. NDF/NDS stands for the ratio of neutral detergent fibre to neutral detergent soluble fractions. Error bars represent standard deviations of three replicate sub-samples and three technical replicates for each section of organic material. X-axis labels refer to the sections analyzed: P8 is the section from the influent to Port 8, P78 is the section from Port 8 to Port 7 and so on.



(a)

Figure S3. Cont.

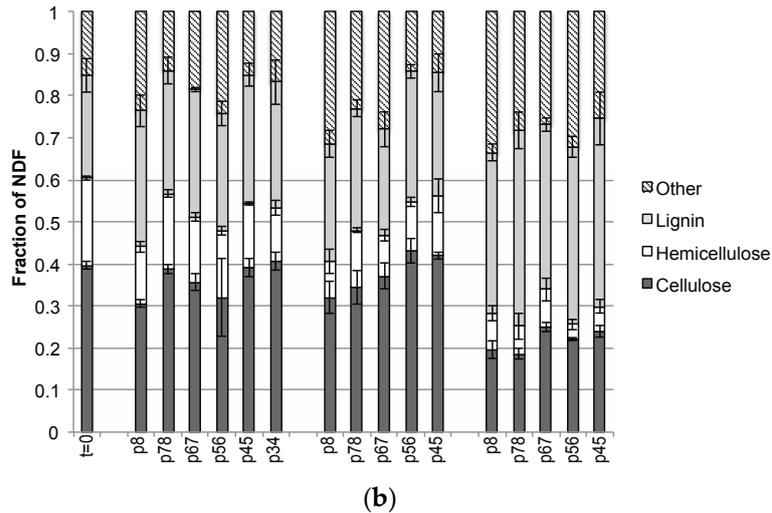


Figure S3. Composition of the neutral detergent fibre fraction in the (a) wood-rich; and (b) hay-rich bioreactors. Other refers to components of the NDF not accounted for as cellulose, hemicellulose or lignin. Error bars represent standard deviations of three replicate sub-samples and three technical replicates for each section of organic material. X-axis labels refer to the sections analyzed: P8 is the section from the influent to Port 8, P78 is the section from Port 8 to Port 7 and so on.

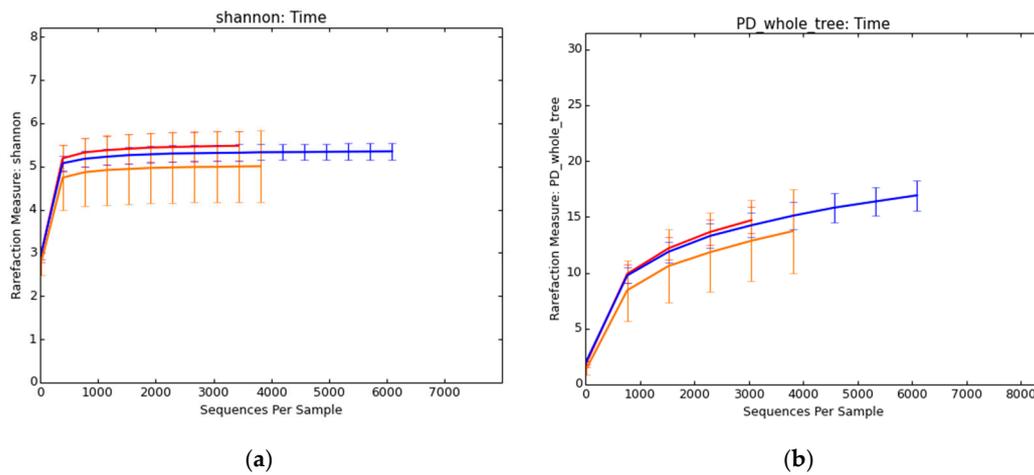


Figure S4. (a) Shannon diversity index; and (b) diversity index based on phylogeny as a function of sequencing depth as determined using rarefaction of the acquired reads. Curves are summarized according to time of sampling with orange representing the time zero samples, blue the early sacrificed samples and red the late sacrificed samples. Overlapping error bars suggest that diversity does not change over time.

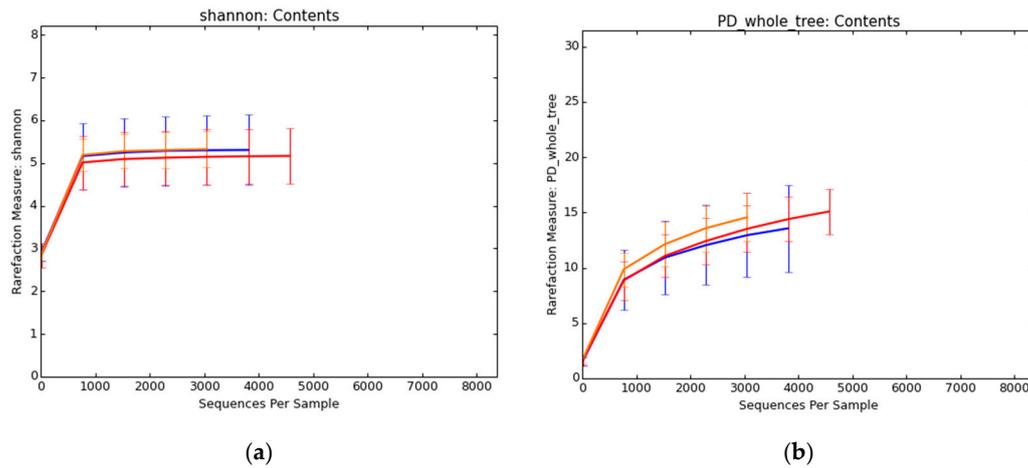


Figure S5. (a) Shannon diversity index; and (b) diversity index based on phylogeny as a function of sequencing depth as determined using rarefaction of the acquired reads. Curves are summarized according to content with blue representing the inocula, orange the wood-rich samples and red the hay-rich samples. Overlapping error bars suggest that diversity is not different between the samples according to their matrix.

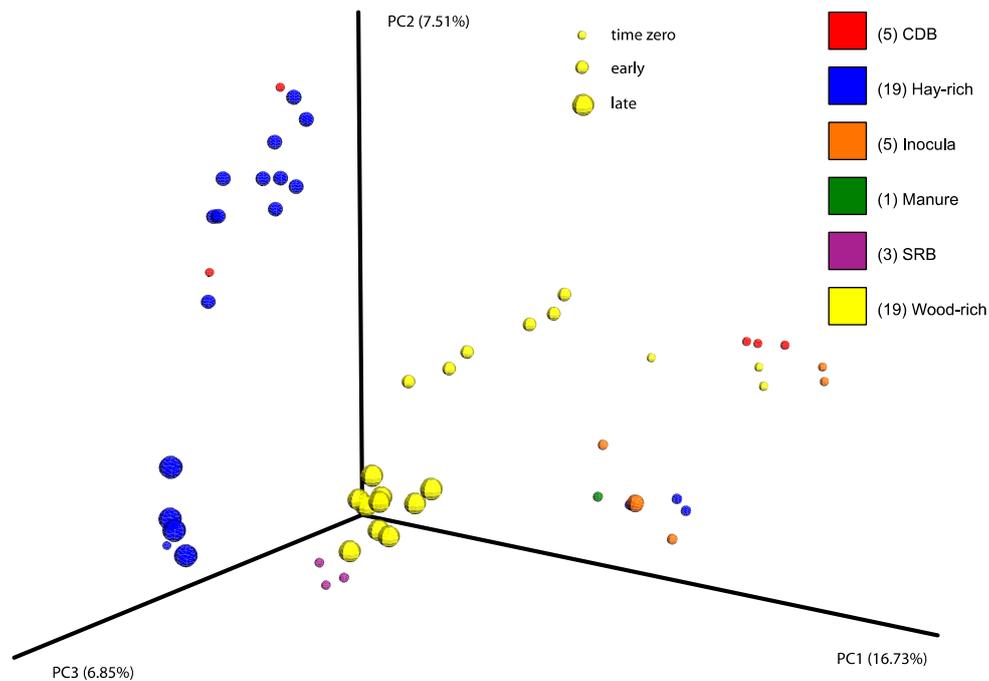


Figure S6. Principle co-ordinate plot of UniFrac distance matrix comparison of microbial communities in the cultures (CDB, SRB and manure), inocula and samples taken at time zero and from the early- and late-sacrificed BCRs. The un-weighted UniFrac matrix was used. Colors depict the type of sample, and size of bubble depicts the time at which the sample was taken.

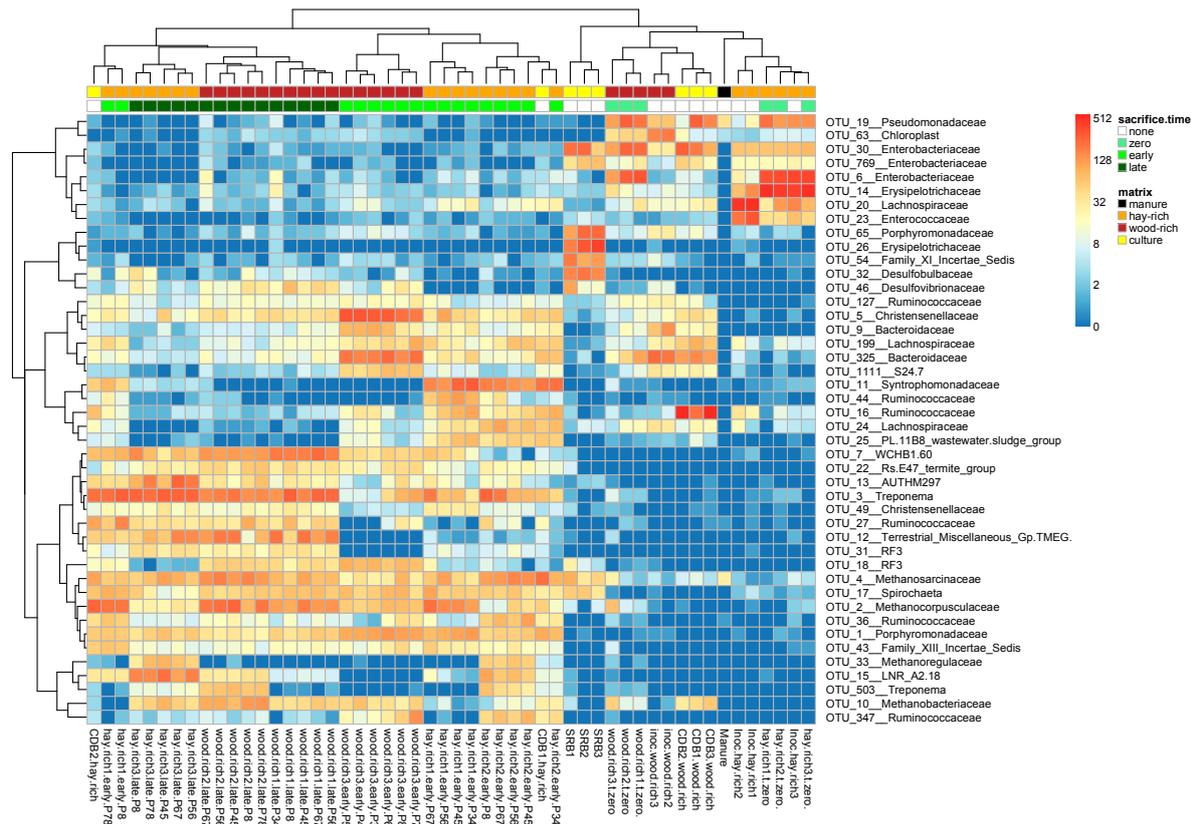


Figure S7. Heatmap of the relative abundances of 90% sequence similarity OTUs in the bioreactors. Samples from the bioreactors are presented as columns and were clustered according to Euclidean distances using hierarchical clustering (hclust in the R programming environment). OTUs are arranged in rows clustered according to Pearson's correlation. Colour intensity represents read counts on a log to the base 2 scale.

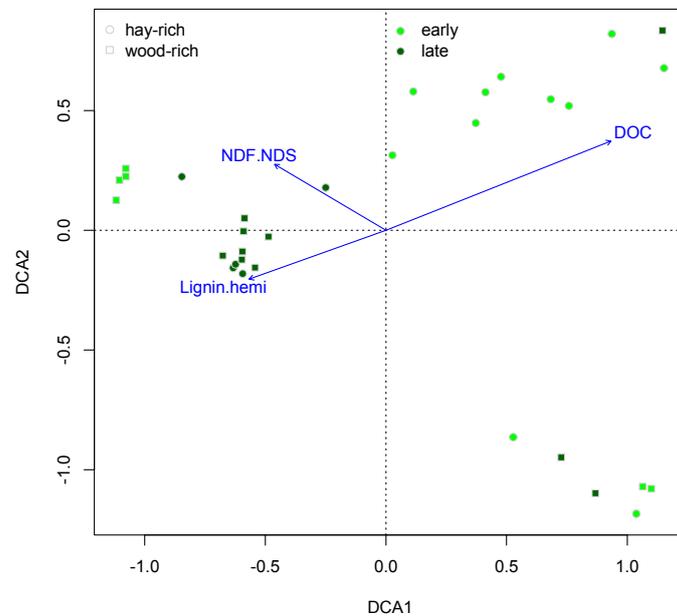


Figure S8. Detrended correspondence analysis (DCA) of the Bray-Curtis dissimilarity comparison of microbial communities in the BCR samples (only BCR samples are shown on this diagram). Samples containing higher concentrations of dissolved organic carbon (indicated in the direction of the blue arrow labeled DOC).

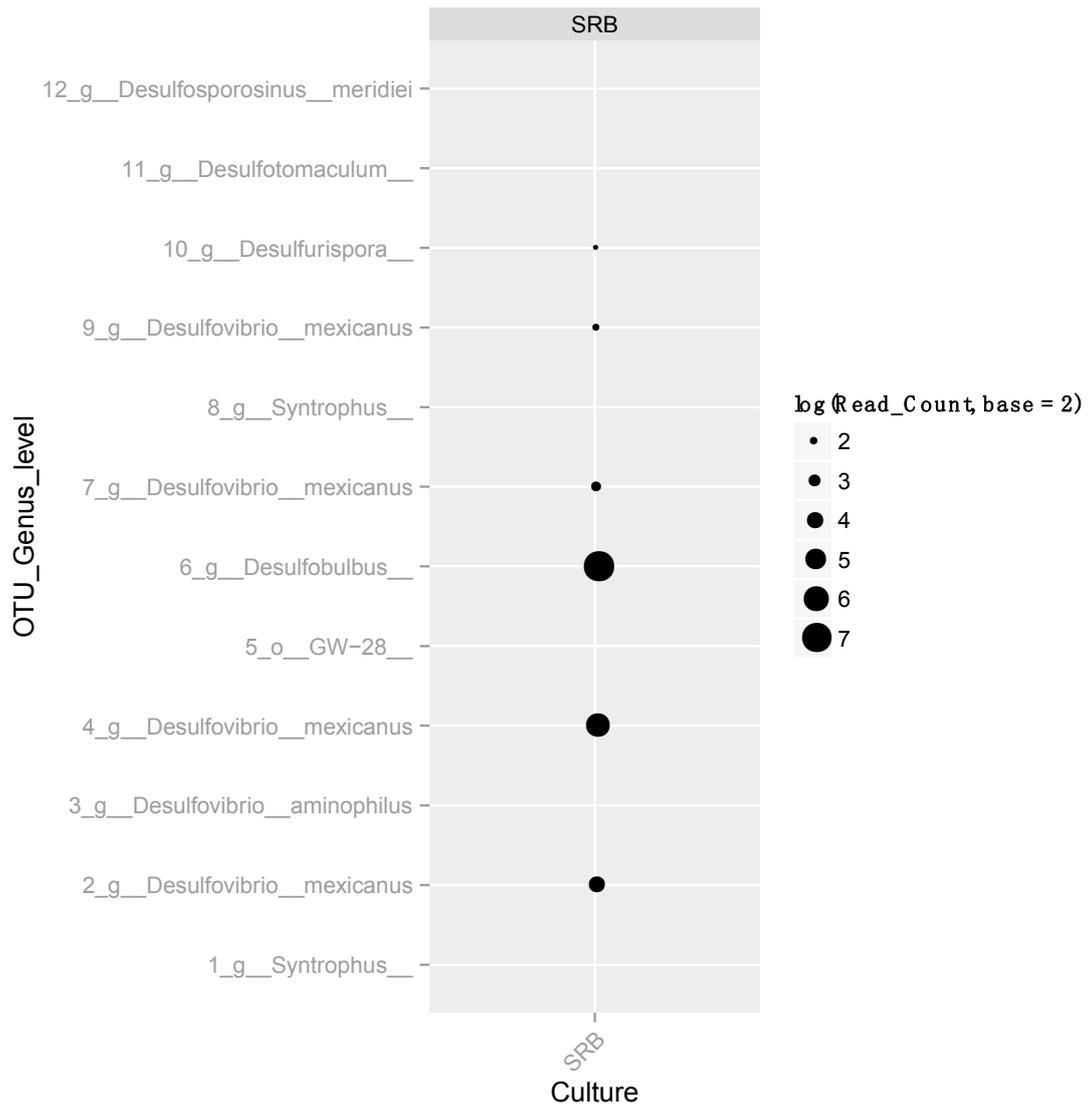


Figure S9. Read counts for sulphate-reducing bacteria related OTUs (94% similarity cut-off) found in the SRB cultures used to prepare the BCR inocula.

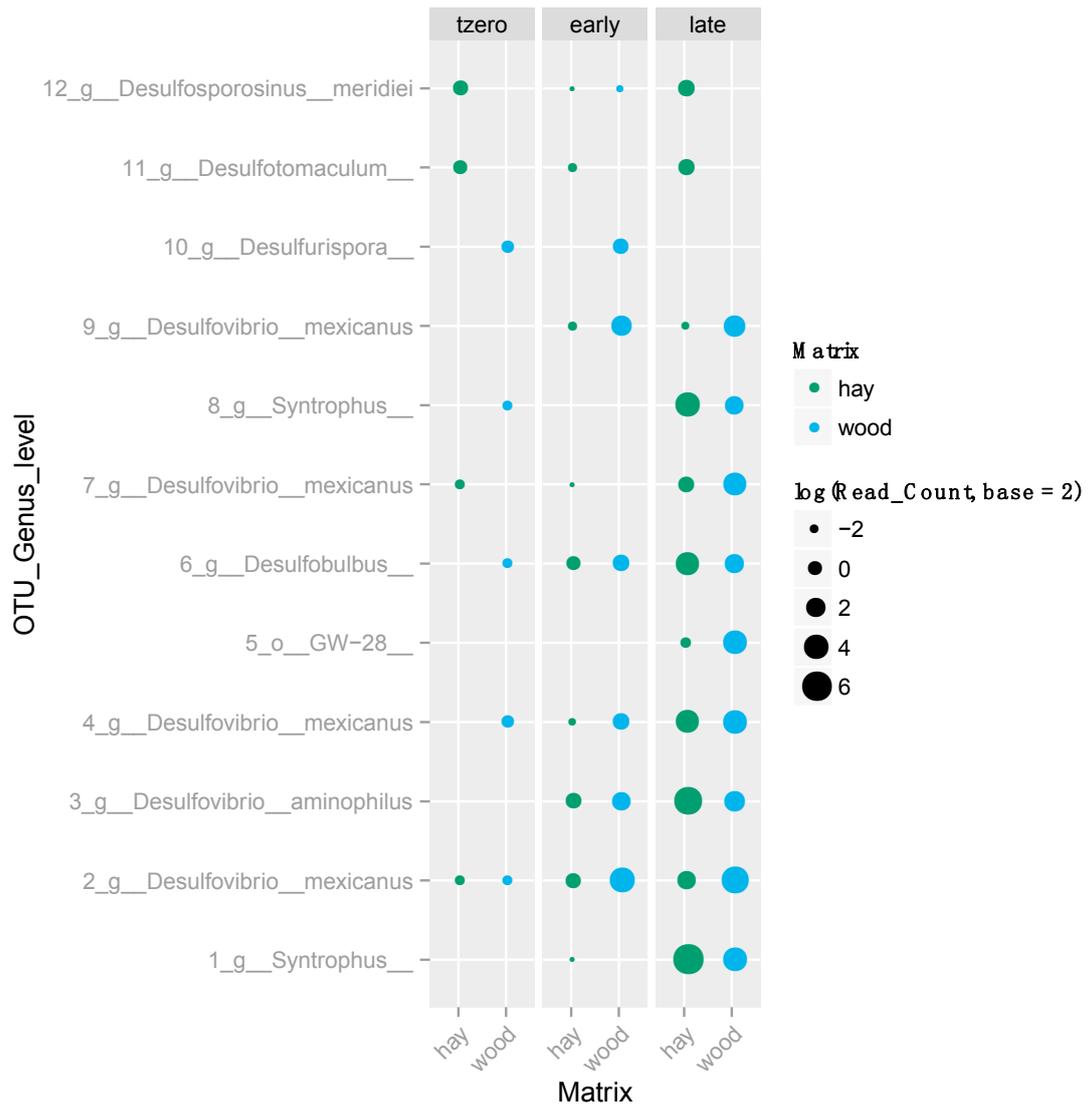


Figure S10. Read counts for sulphate-reducing bacteria related OTUs (94% similarity cut-off) found in the SRB cultures used to prepare the BCR inocula.