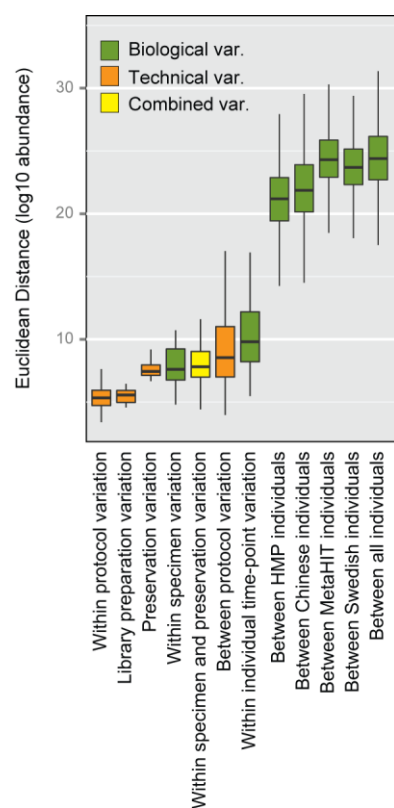
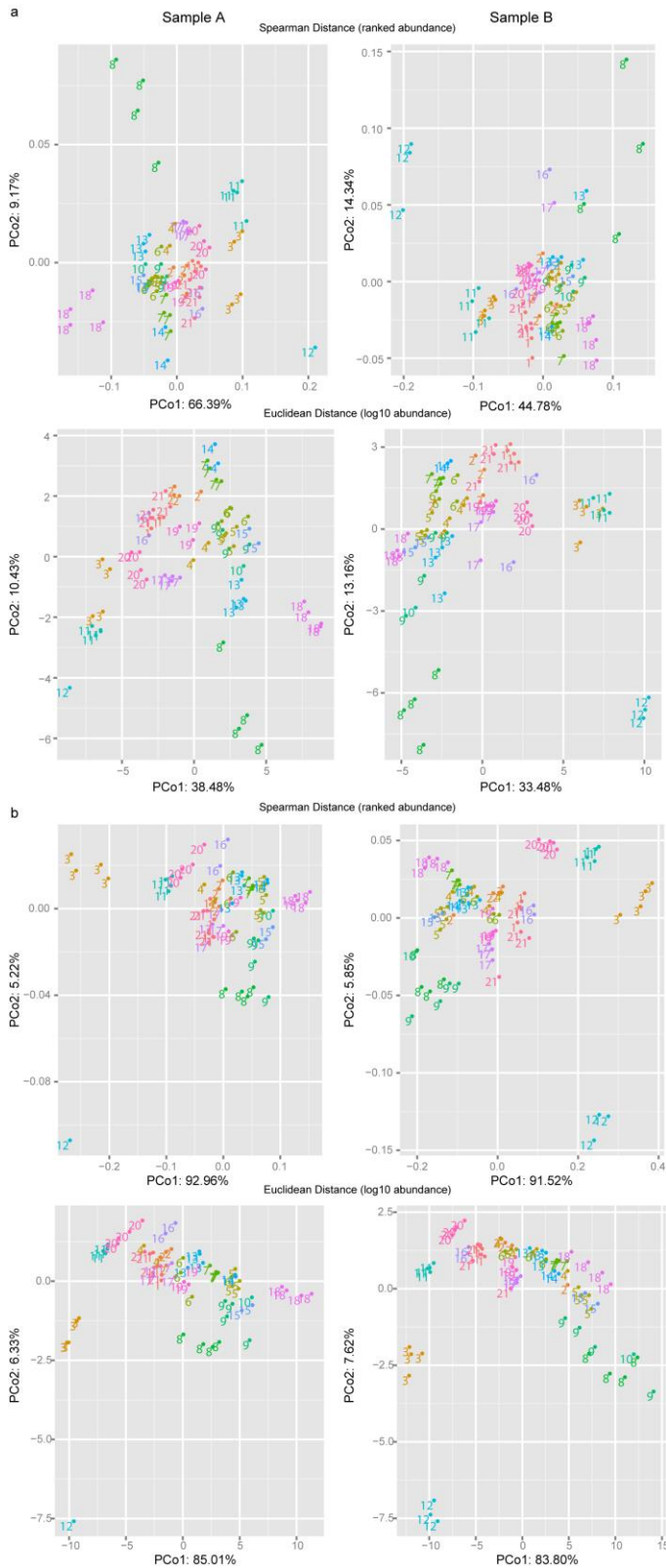


Supplementary Information



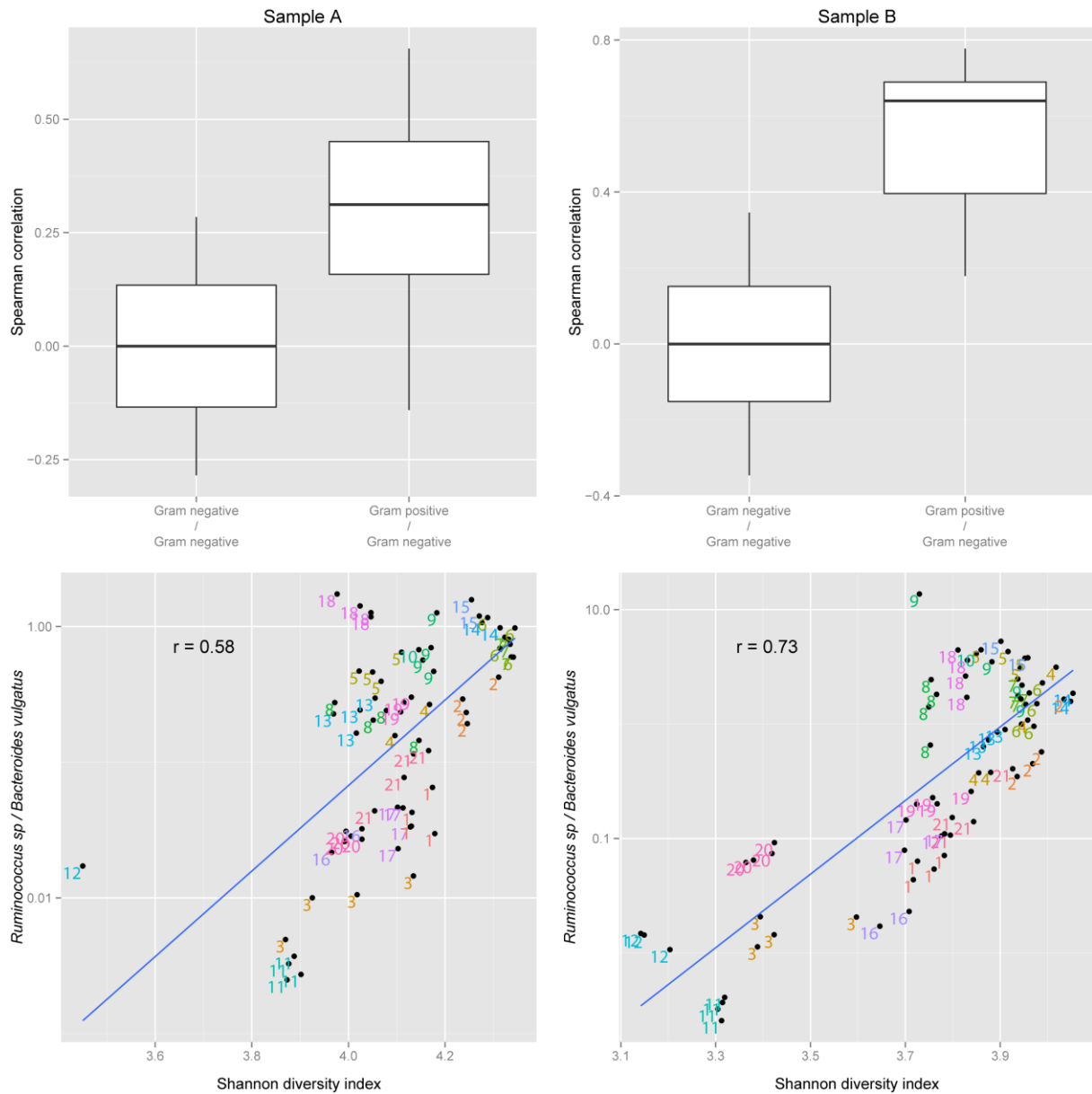
Supplementary Figure 1: Inter-individual distance dependence on study.

Similar to Figure 3, we show the estimated effect sizes of different parameters in the context of inter-individual distance assessed within the different studies used. It is clear that while small, there are clear differences in the median distance within studies, with HMP samples appearing to be more homogenous than MetaHIT ones.



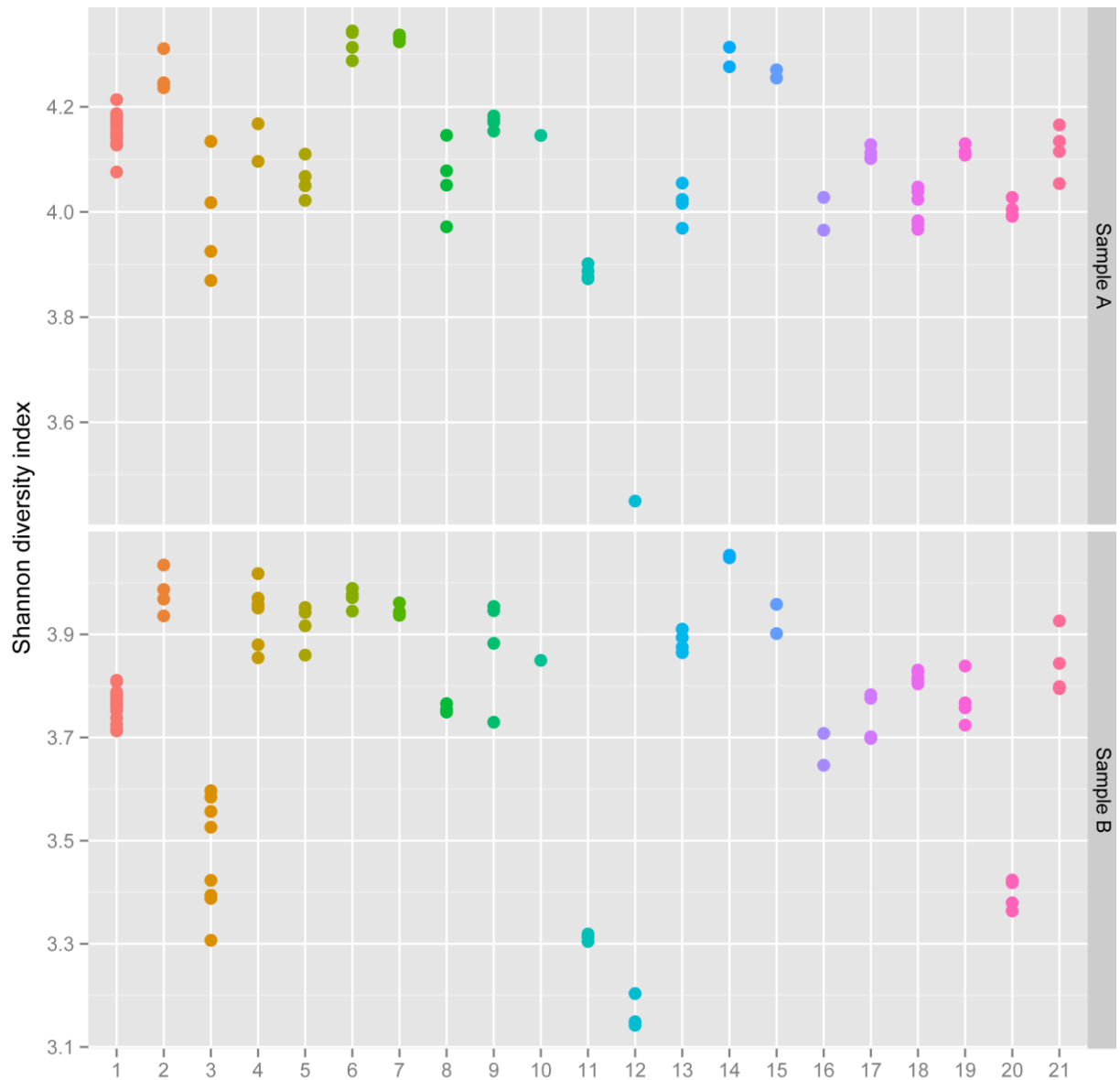
Supplementary Figure 2: Extraction bias across the two samples.

Extraction bias is consistent across the two samples, independent of the distance measure that was used. (a) shows a PCoA projection of the species abundances for each sample, independently, using a Spearman ranked correlation as well as a Euclidean distance. Most of the variation is captured by the first two principal coordinates and the clustering of extraction methods is easily observable. (b) shows a PCoA projection of the functional distance, both Spearman ranked and Euclidean.



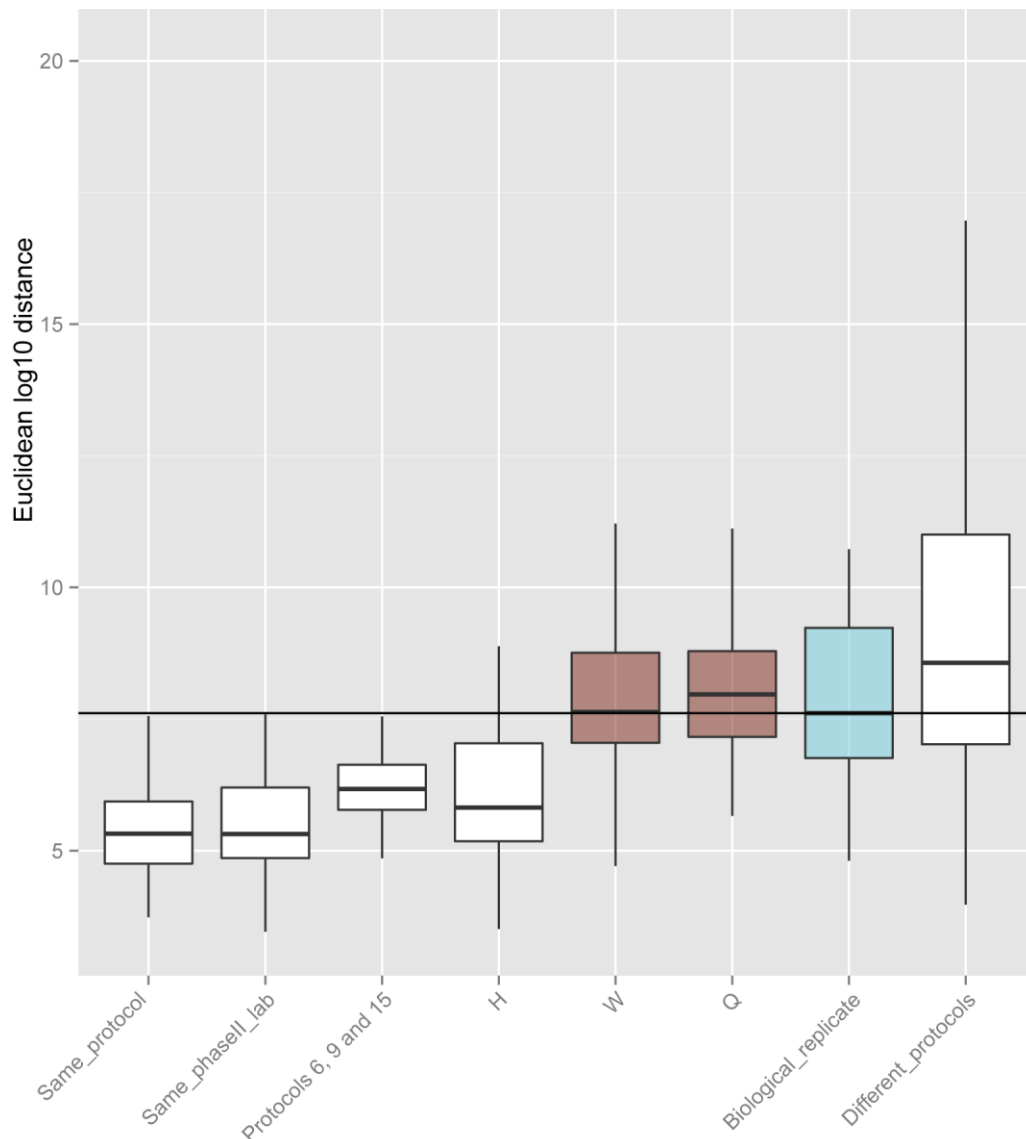
Supplementary Figure 3: Lysis of Gram-positive bacteria positively correlates with Shannon diversity.

Recovery of Gram-positive bacteria correlates with overall Shannon diversity. Considering only the top 20 most abundant species within each sample, ratios were computed between all Gram-positive and Gram-negative bacteria as well as Gram-negative to Gram-negative bacteria. The top panel shows the correlation of these ratios with the Shannon diversity index, while the lower panel exemplifies this correlation on the most abundant Gram-positive and Gram-negative bacteria that are common to both samples A and B, indicating the strong positive relation between recovery of Gram-positive bacteria and observed Shannon diversity.



Supplementary Figure 4: Shannon diversity of sample composition

Observed Shannon diversity is consistently influenced by extraction method, as illustrated in both samples. Furthermore, there is a considerable difference in diversity between the two samples, which is not overwritten by extraction bias.



Supplementary Figure 5: Extraction bias of best performing protocols considered in Phase II

Extraction variation is the same in Phase II replicates as that of Phase I (bars 1 and 2, respectively). Furthermore, the three protocols that have been merged into protocol Q for Phase II, namely 6, 9 and 15 produce similar results and present extraction bias below the biological replicate variation. The tree Phase II protocols (H, W and Q), when applied in different laboratories, with no previous experience in the particular protocol used, produce comparable abundance estimates, with errors below the level of biological variation within one specimen.

Supplementary Tables

Supplementary Table 1: Protocol descriptors

A condensed version of all extraction protocols used. A detailed description of each can be found in Supplementary Information.

Supplementary Table 2: Members and composition of mock community

Contains the identifiers of all 10 bacterial species that make up the mock, together with their respective abundance measured in duplicate by FACS and OD.

Supplementary Table 3: Sample description

This table describes the relation between sample names and extraction protocols, for all samples across all phases.