# Impact of sequencing depth on the characterization of the microbiome and resistome.

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## Supplementary information (Table S1, Figures S1 – S6):

Table S1: DNA concentration (PicoGreen) and purity (NanoDrop) for samples submitted for sequencing.

| Sample ID | Concentration (PicoGreen) (ng/µl) | Total quantity (µg) | 260/280 ratio (NanoDrop) |
|---|---|---|---|
| N**003** | 78.24 | 3.91 | 1.78 |
| N**013** | 50.69 | 2.53 | 1.82 |
| S**034** | 79.68 | 3.98 | 1.85 |
| S**040** | 59.00 | 2.95 | 1.84 |
| V**046** | 87.17 | 4.36 | 1.87 |
| V**053** | 89.09 | 4.45 | 1.87 |
| A**062** | 92.03 | 4.60 | 1.84 |
| A**070** | 104.26 | 5.21 | 1.84 |

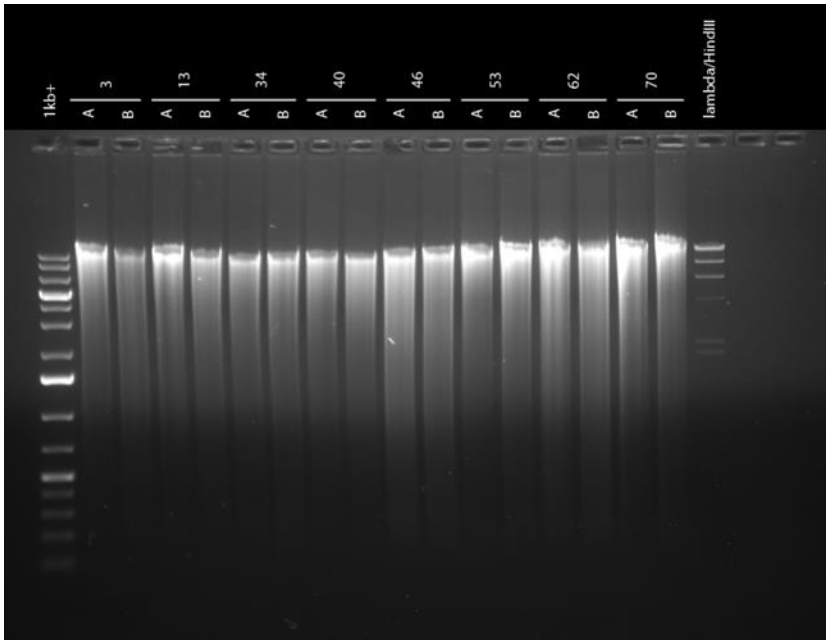
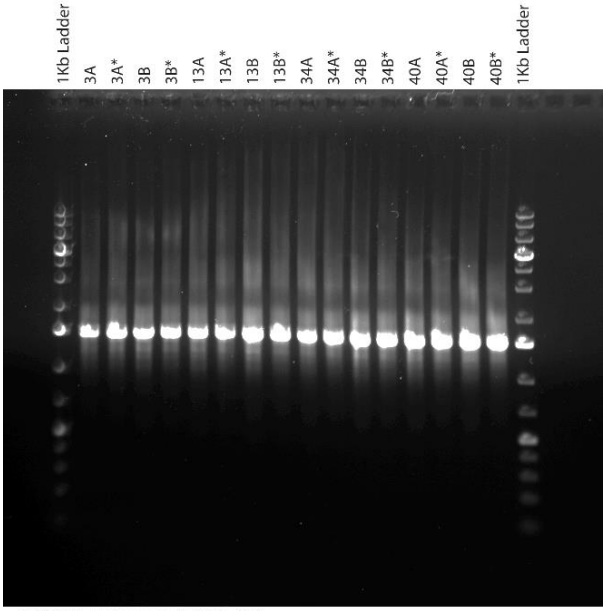
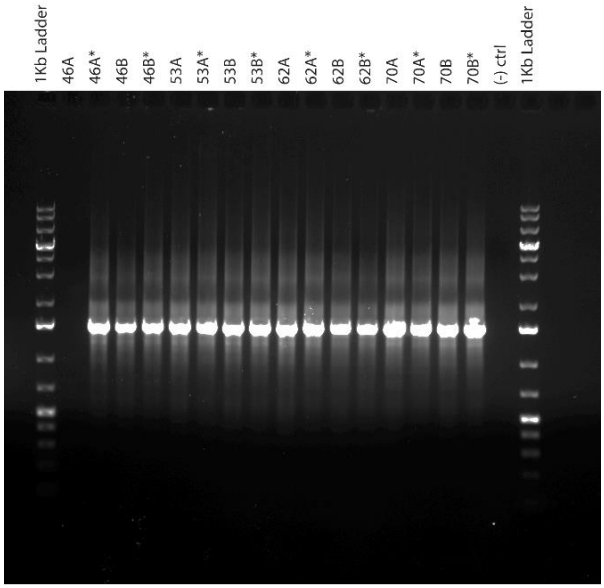



**Figure S1**: Gel electrophoresis of metagenomic DNA isolated. Loaded 4 µL on 1% agarose gel; run at 105V for 1 hr. Replicate samples A & B were pooled prior to submitting samples for sequencing.




SAMPLES: metagenomic DNA_16S
(*)=1/10 dilution of pure sample
PCR conditions= 4min:95C, 30sec: 94C, 30sec:58C, 1:30sec:72C, 10min:72C,
HOLD:6C: x35 cycles
GEL: 1% agarose,75mL, 30wsell comb, 1Kb Plus Ladder, 5uL loaded, 1hr@100V
Expected product size: ~1.4Kb

**A**

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Expected product size: ~1.4Kb

**B**

**Figure S2 A & B**: Control PCR targeting bacterial 16SrRNA gene (27F and 1492R primer pair). Both undiluted and diluted samples could be appropriately amplified indicating that DNA was free of PCR inhibitors.

NOTE: No sample was loaded in lane 46A in Figure S2 B.

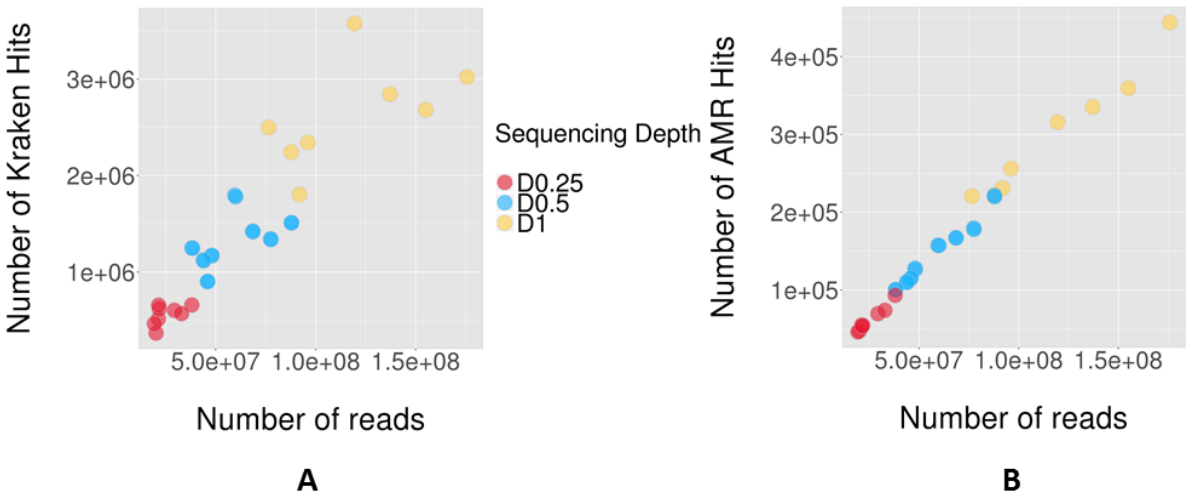
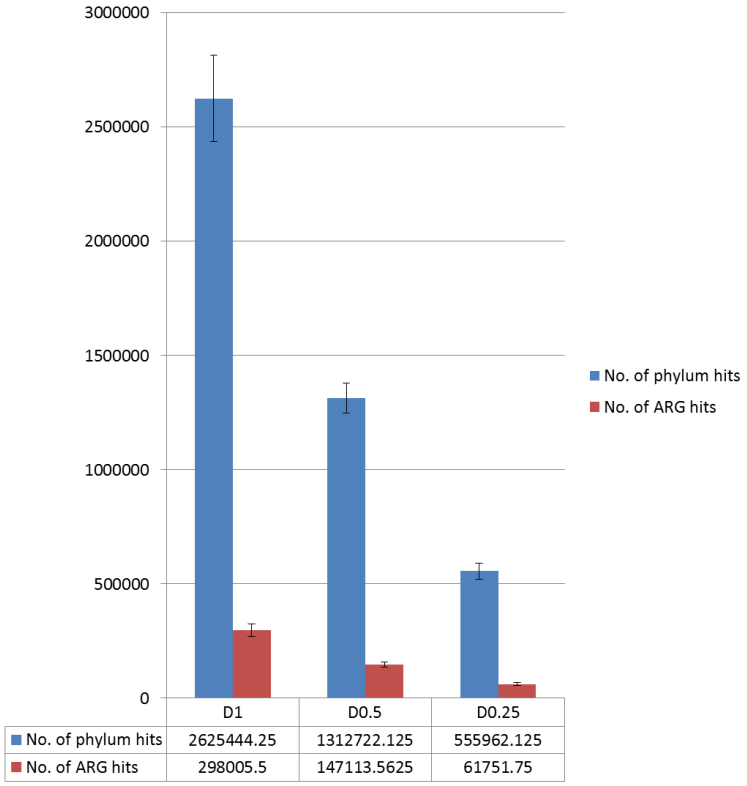



**Figure S3:** Correlation between A) numbers of sequence reads and taxonomic assignments as identified by Kraken, and B) numbers of sequence reads and assignments to the MEGARes AMR database for D1 (n=8), D0.5 (n=2 x 8; 16 total) and D0.25 (n=8) samples. Values of duplicate samples in D0.5 dataset overlap, hence appearing as single dot.




| | D1 | D0.5 | D0.25 |
|---|---|---|---|
| No. of phylum hits | 2625444.25 | 1312722.125 | 555962.125 |
| No. of ARG hits | 298005.5 | 147113.5625 | 61751.75 |

**Figure S4:** The average number of reads aligning to phyla and to the MEGARes AMR database at various sequencing depths increased on by ~2.4 fold for D0.5 compared to D0.25 and by ~2 fold for D1 compared to D0.5.

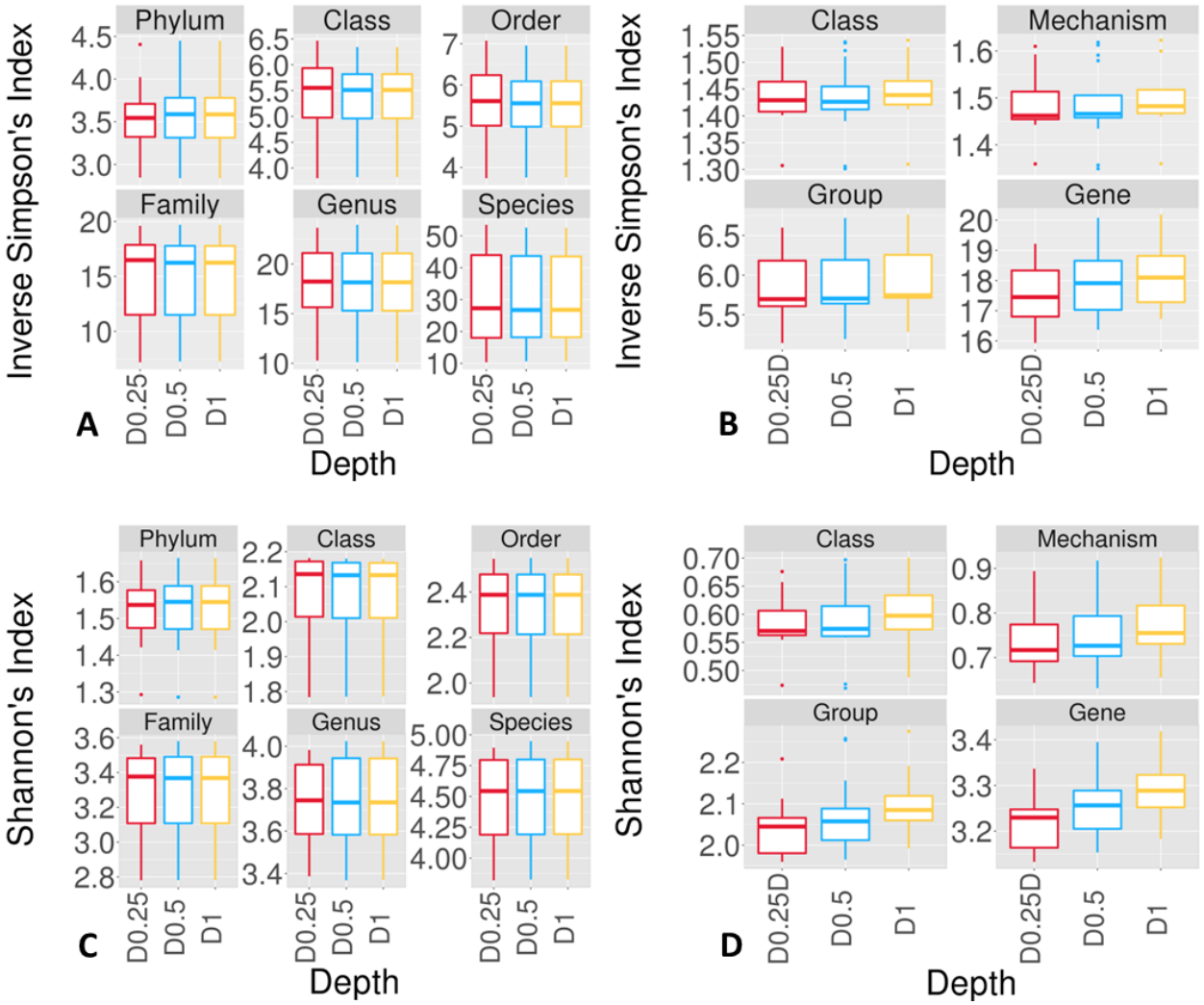



**Figure S5: α-diversities of microbiome and resistome at various sequencing depths.** Box-and-whisker plots showing A) microbial taxon α-diversity Inverse Simpson's index; B) AMR category α-diversity Inverse Simpson's index; C) microbial taxon α-diversity Shannon's index; D) AMR category α-diversity Shannon's index. Boxes represent the interquartile ranges (upper line is the 75% quantile, and the lower line is the 25% quantile), the lines inside the boxes are the medians, the whiskers span the range of the 25% quantile or the 75% quantile plus 1.5 times the interquartile range, and dots are outliers.

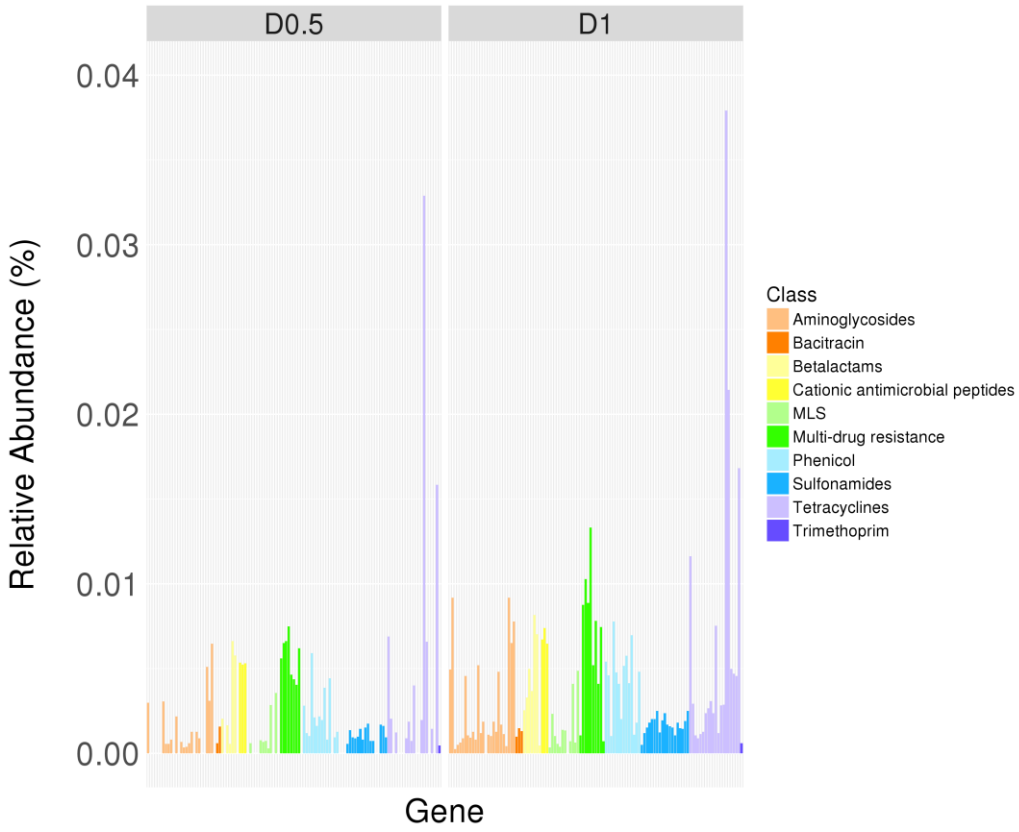



**Figure S6:** Relative abundance of ARGs missing in the D0.25 dataset but present in higher depth datasets D1 & D0.5.