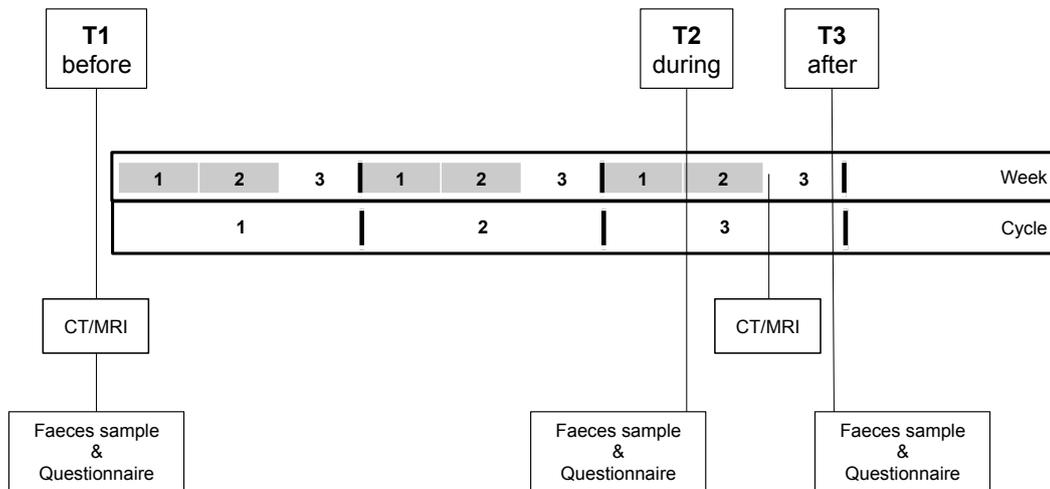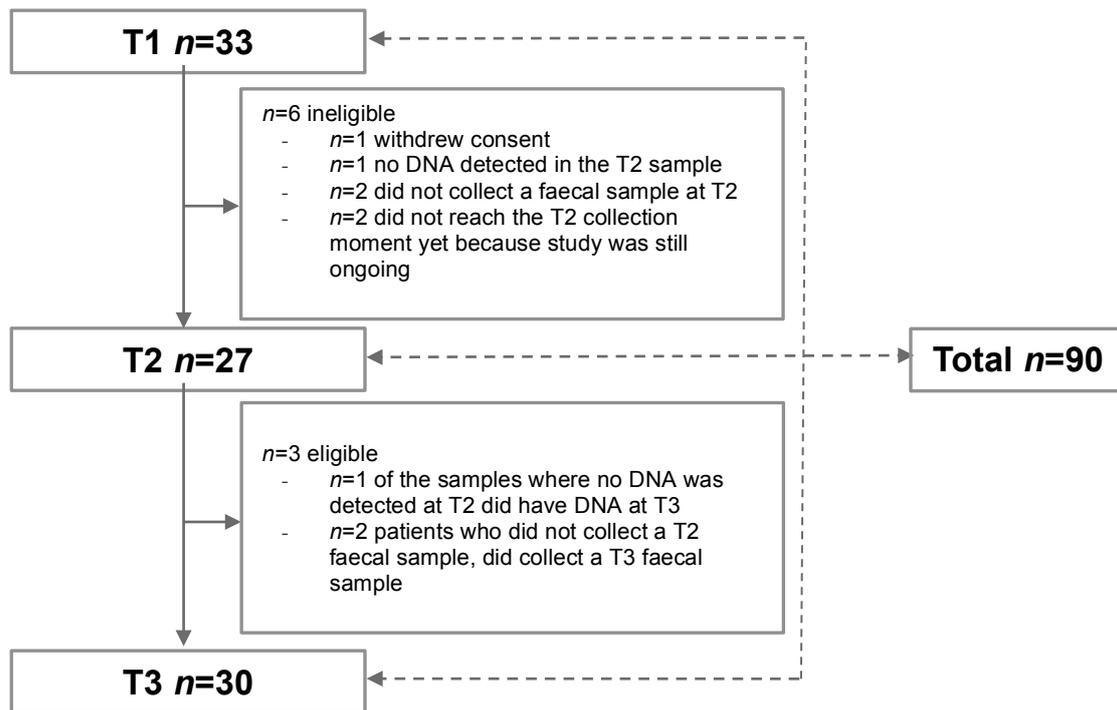# Supplementary figures

Figure S1: Study design.
Patients collected faecal samples and completed questionnaires at three time points. **T1** is collected before the start of the first capecitabine cycle, **T2** is collected between day 7-14 of the third cycle, and T3 is collected at day 20 or 21 of the third cycle. Tumour response was assessed using CT or MRI scans before and at the end of three cycles capecitabine by means of RECIST. During the observation period, patients received three cycles capecitabine (1000-1250 mg/m^2 orally, twice daily on days 1-14 in a three week cycle), this is illustrated in gray. Depending on the decision of the medical oncologist, patients continue with capecitabine treatment after the third treatment cycle.
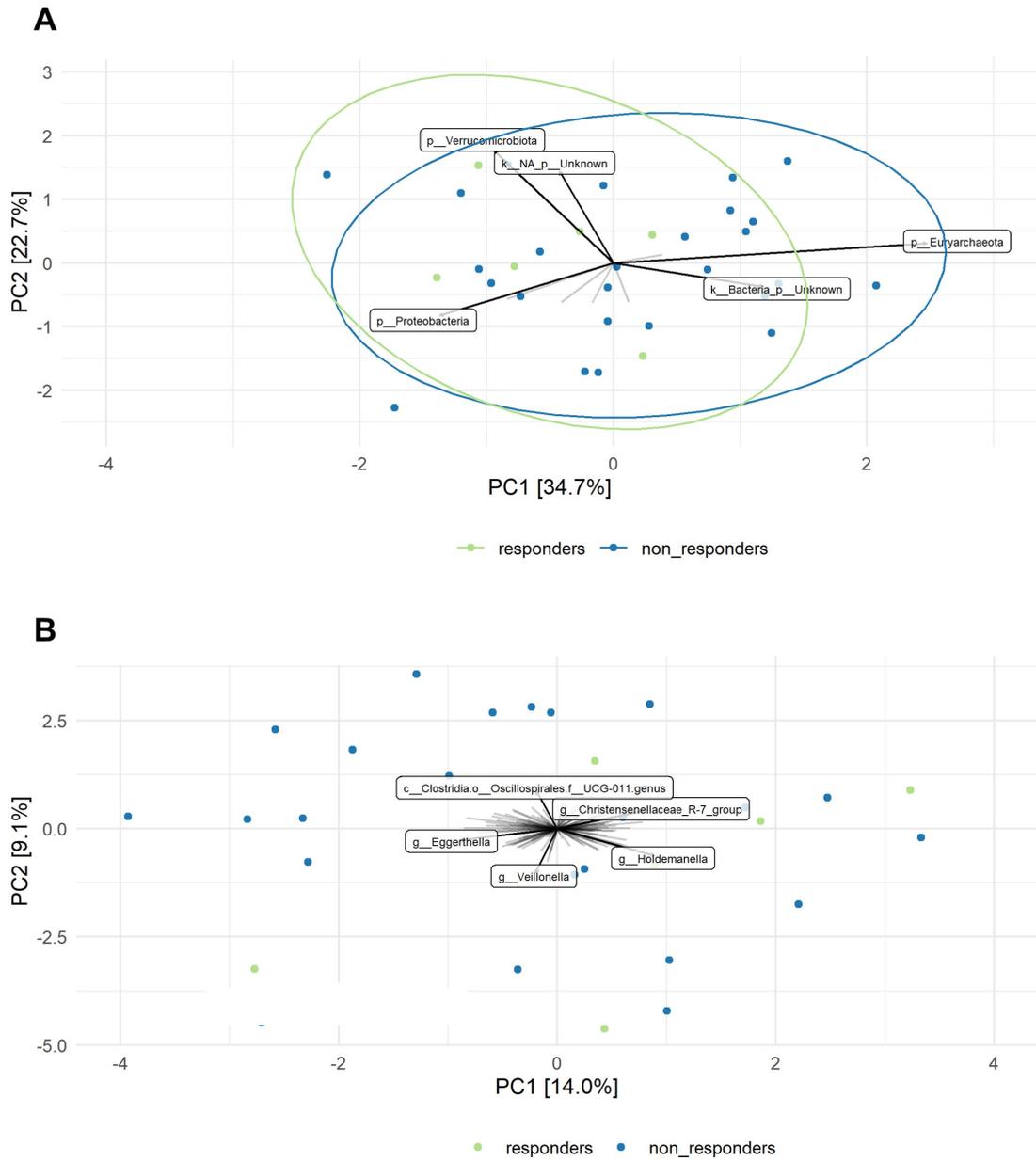
```
┌────────────────┐                                    ┌ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─
│  T1 n=33       │◄─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ┐
└────────────────┘                                    │
        │         ┌──────────────────────────────────────┐
        │         │ n=6 ineligible                       │
        │         │   -  n=1 withdrew consent            │
        ├────────►│   -  n=1 no DNA detected in the T2 sample │
        │         │   -  n=2 did not collect a faecal sample at T2 │
        │         │   -  n=2 did not reach the T2 collection │
        │         │      moment yet because study was still │
        │         │      ongoing                         │
        │         └──────────────────────────────────────┘
        ▼
┌────────────────┐                                        ┌────────────────┐
│  T2 n=27       │◄ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─►│  Total n=90    │
└────────────────┘                                        └────────────────┘
        │         ┌──────────────────────────────────────┐
        │         │ n=3 eligible                         │
        │         │   -  n=1 of the samples where no DNA was │
        │         │      detected at T2 did have DNA at T3 │
        ├────────►│   -  n=2 patients who did not collect a T2 │
        │         │      faecal sample, did collect a T3 faecal │
        │         │      sample                          │
        │         └──────────────────────────────────────┘
        ▼
┌────────────────┐
│  T3 n=30       │◄ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ─ ┘
└────────────────┘
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Figure S2: Overview of faecal samples used for 16S rRNA gene sequencing of the V4 hypervariable region.
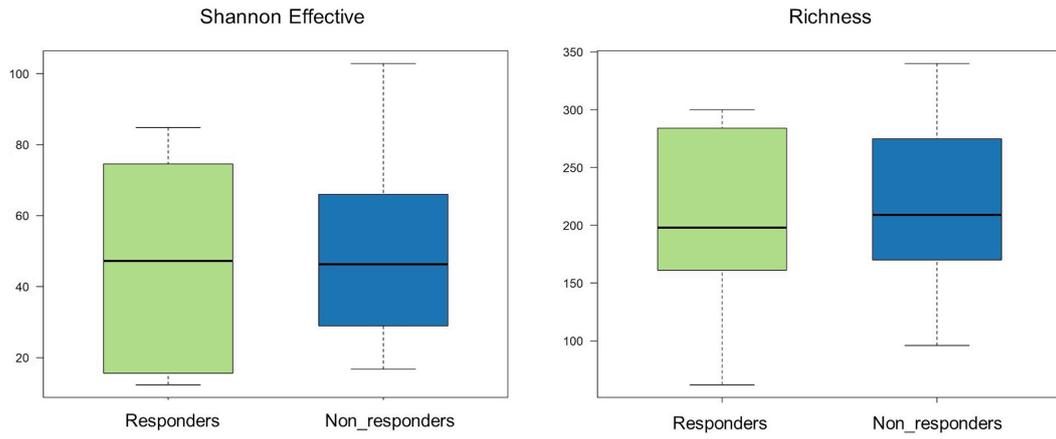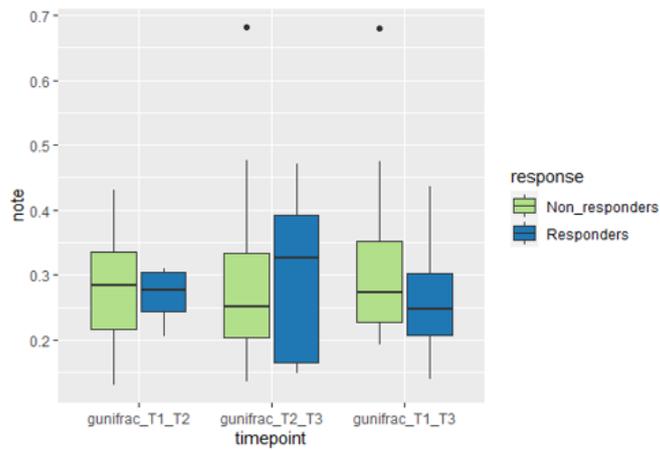
Figure S3: α−diversity measures.
Microbial diversity and richness of responders and non-responders at T1, measured in terms of Shannon effective (*p*=0.786) and observed richness (*p*=0.528) (Table S9). Numbers presented in median (IQR).
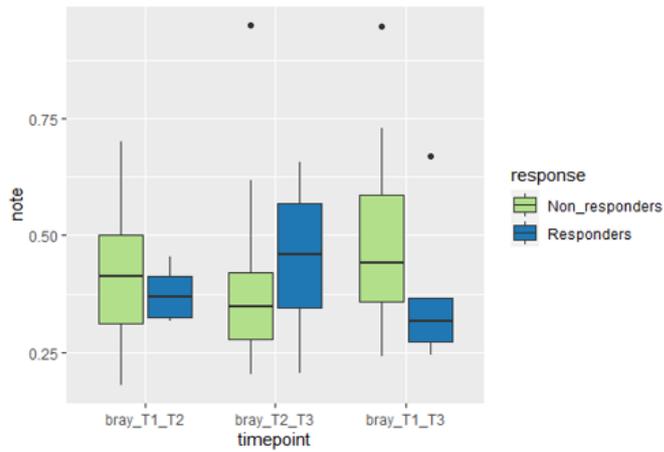
A

PC2 [22.7%]

p__Verrucomicrobiota
k__NA_p__Unknown
p__Euryarchaeota
k__Bacteria_p__Unknown
p__Proteobacteria

PC1 [34.7%]

responders   non_responders

B

PC2 [9.1%]

c__Clostridia.o__Oscillospirales.f__UCG-011.genus
g__Christensenellaceae_R-7_group
g__Eggerthella
g__Holdemanella
g__Veillonella

PC1 [14.0%]

responders   non_responders

Figure S4: Ordination plots derived from unconstrained Principal Components Analysis (PCA), showing overall composition of the microbial community on phylum (A) and genus level (B) at T1. Aitchison distance was used. 10 phyla and 156 genera were included for this analysis. Data were transformed using centre-log-ratio transformation. Names are given for genera which contributed most to overall microbial variation.

Figure S5: Temporal (in)stability in microbial communicty structure (β-diversity).
Changes of generalized UniFrac distances (A) and Bray-Curtis distances (B) between T1 versus T2, T2 versus T3 and T1 versus T3. At all time points, distances were not significantly different between responders and non-responders.