Supplemental methods

Assignment of taxonomic annotation and predicted metagenomics

MiSeq FastQ files were created using CASAVA 1.8.2 (https://support.illumina.com/sequencing/sequencing_software/casava). The open-source software package DADA2[1] (v.1.10) was used for amplicon-data processing which enables a single-nucleotide resolution of amplicons (amplicon sequence variants, ASVs). Data processing was conducted according to the recommended procedure for large datasets (https://benjjneb.github.io/dada2/bigdata.html), adapted to the targeted V1-V2 amplicon. In brief, five bases were truncated from the 5' end of the sequence of both reads. Forward and reverse reads were truncated to a length of 200 and 150 bases, respectively. If the sequence quality dropped below a quality score of five, a shorter resulting read length after truncation was also possible. Exclusion of read-pairs was performed if they contained ambiguous bases, had expected errors higher than two or when originating from PhiX spike-in. To infer error profiles, 1 million reads of the respective sequencing run were used. Subsequently dereplication, error correction and merging of forward and reverse reads was conducted. The removeBimeraDenovo() function in consensus mode was used to combine ASV abundance of tables of all samples and to identify and remove chimeric amplicon sequences. For assignment of taxonomic annotation a Bayesian classifier and the Ribosomal Database Project training set (v.16) were used. The functional genomic potential of the faecal microbiota were predicted with PICRUSt2[2] using the standard workflow as described at https://github.com/picrust/picrust2/wiki/Workflow and ASVs from the DADA2 pipeline as input. All samples were normalized to 10,000 *16S rRNA*-gene read counts for analysis.

REFERENCES

1 Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* 2016;13(7):581–83.
2 Douglas GM, Maffei VJ, Zaneveld JR, et al. PICRUSt2 for prediction of metagenome functions. *Nat Biotechnol* 2020;38(6):685–88.