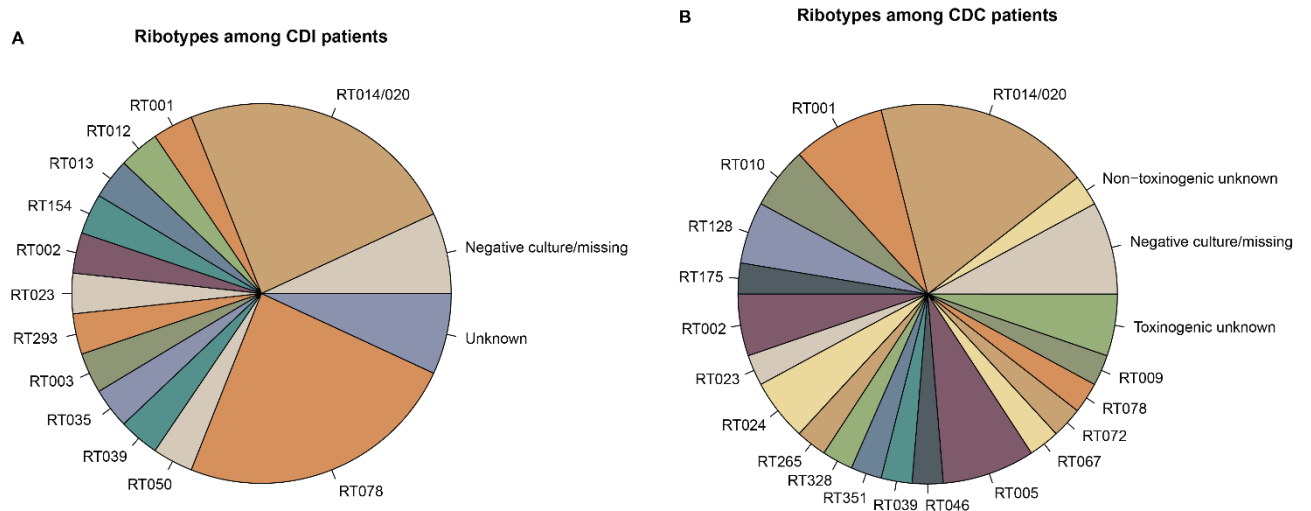
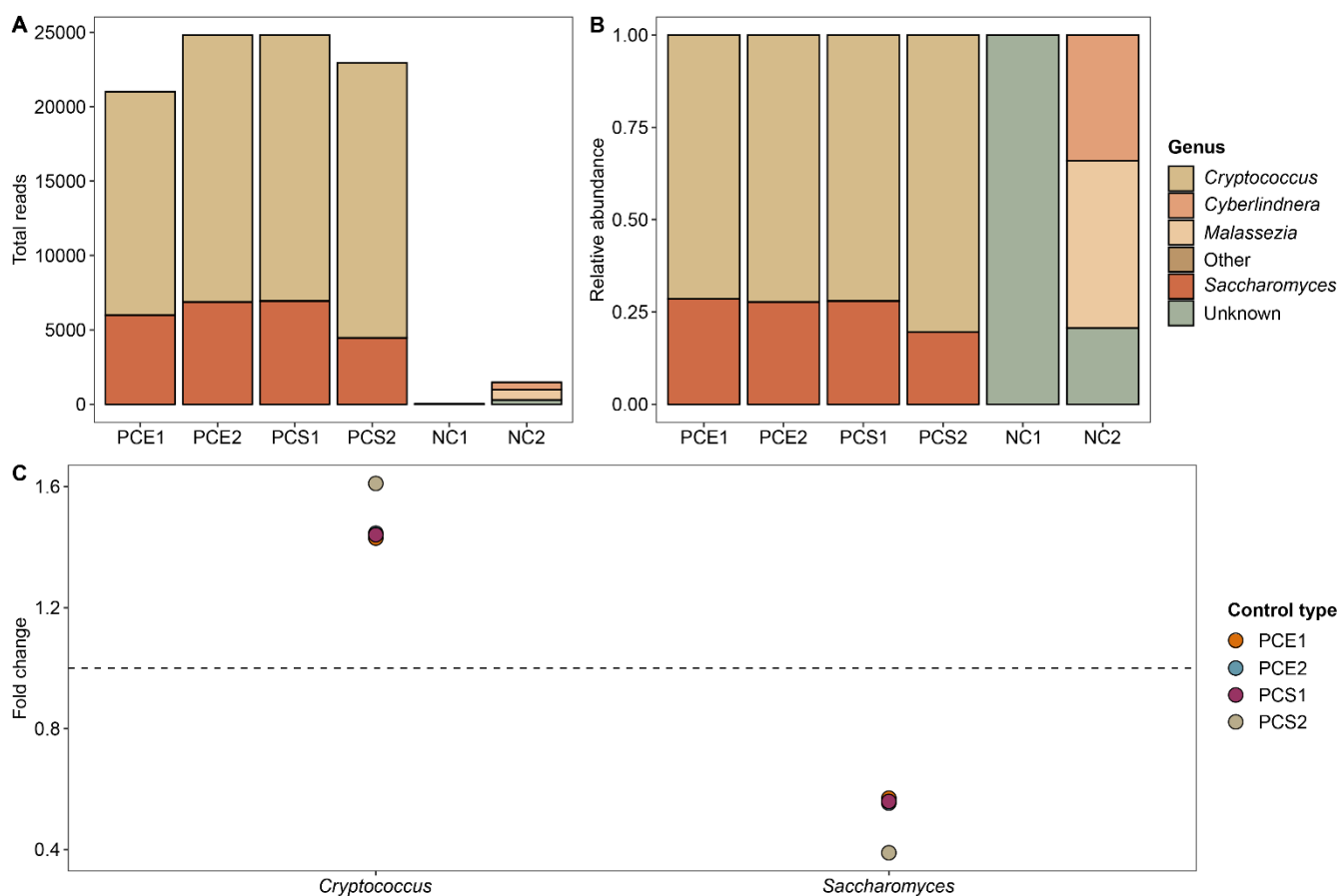


- 1 **Supplementary Material: Fungal and bacterial gut microbiota differ between**
- 2 ***Clostridioides difficile* colonization and infection**

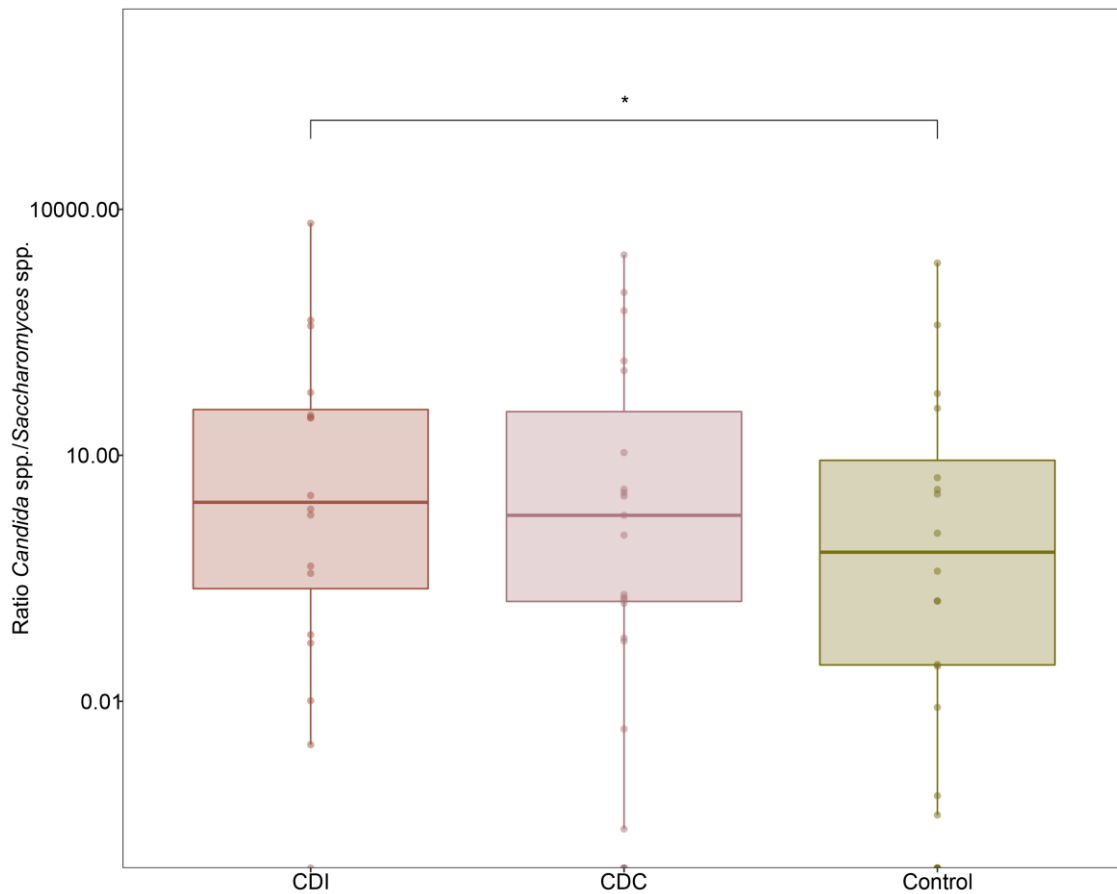


3 **Supplementary Figure 1.** Distribution of ribotypes among (A) CDI patients ($n = 29$)
 4 and (B) asymptomatic CDC patients ($n = 38$). Ribotyping was performed with capillary
 5 PCR. Ribotype 078 was significantly more prevalent in CDI (24.10%) compared to
 6 CDC (2.63%), $P = 0.017$ (Fisher’s Exact Test) This figure is adapted with permission
 7 from Crobach *et al.* published in *Microorganisms*^[29]. CDC: *C. difficile* colonization;
 8 CDI: *C. difficile* infection.



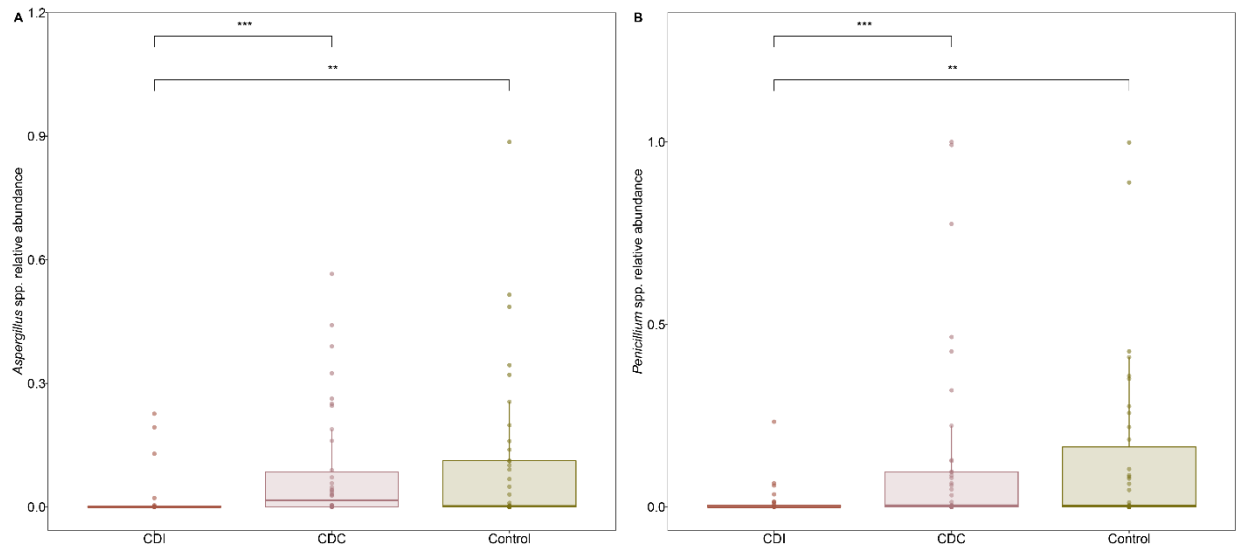
9 **Supplementary Figure 2.** Positive and negative DNA extraction controls (PCE and
 10 NC) and positive sequencing controls (PCS). Fungal taxonomic composition of the five
 11 most abundant genera shown as total reads (A) and as relative abundance (B) Taxa not
 12 belonging to the five most abundant genera were summarized into “Other”; (C) Fold
 13 change in the relative abundance of the two fungal mock genera compared to the
 14 theoretical relative abundance in the mock community (horizontal line).

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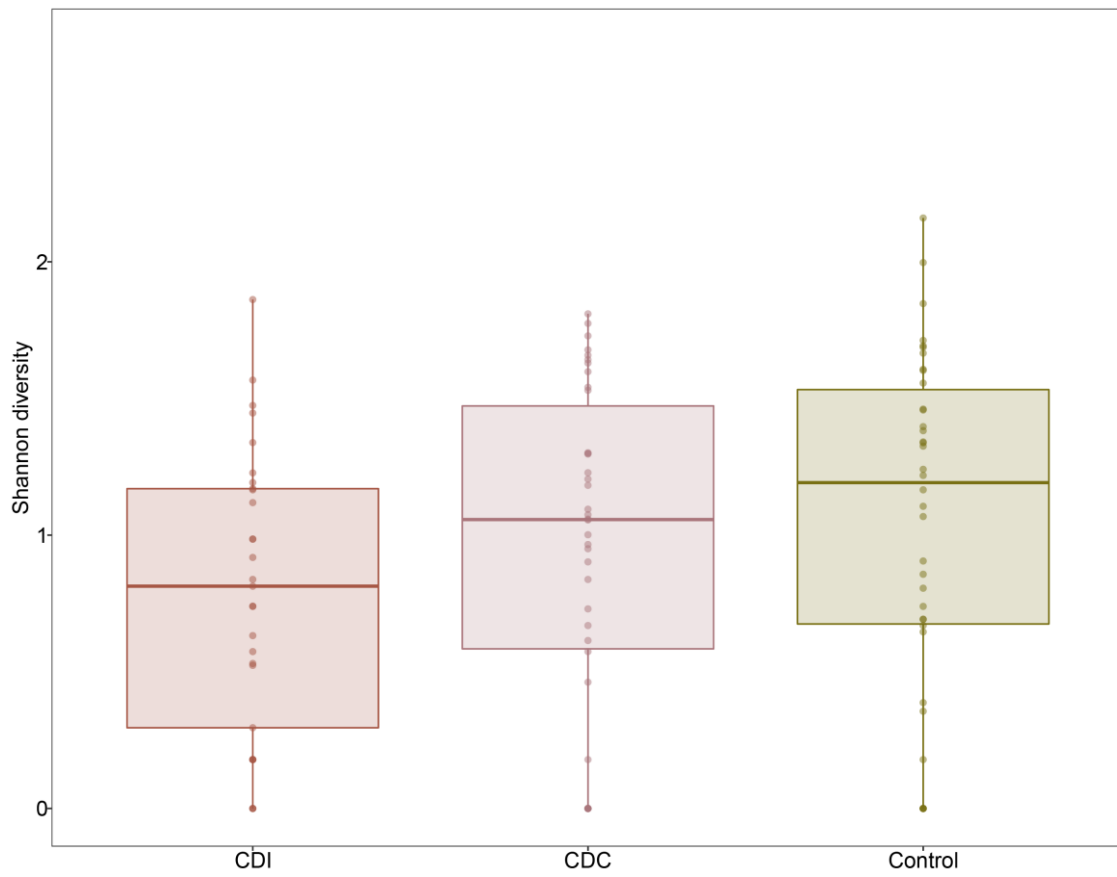


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17 **Supplementary Figure 3.** Logarithmic scale of the ratio *Candida* spp. to
 18 *Saccharomyces* spp. in CDI patients, asymptomatic CDC patients and controls
 19 (Control). * $P \leq 0.05$ (Wilcoxon Rank Sum Test). Non-significant results are not
 20 displayed. CDC: *C. difficile* colonization; CDI: *C. difficile* infection.

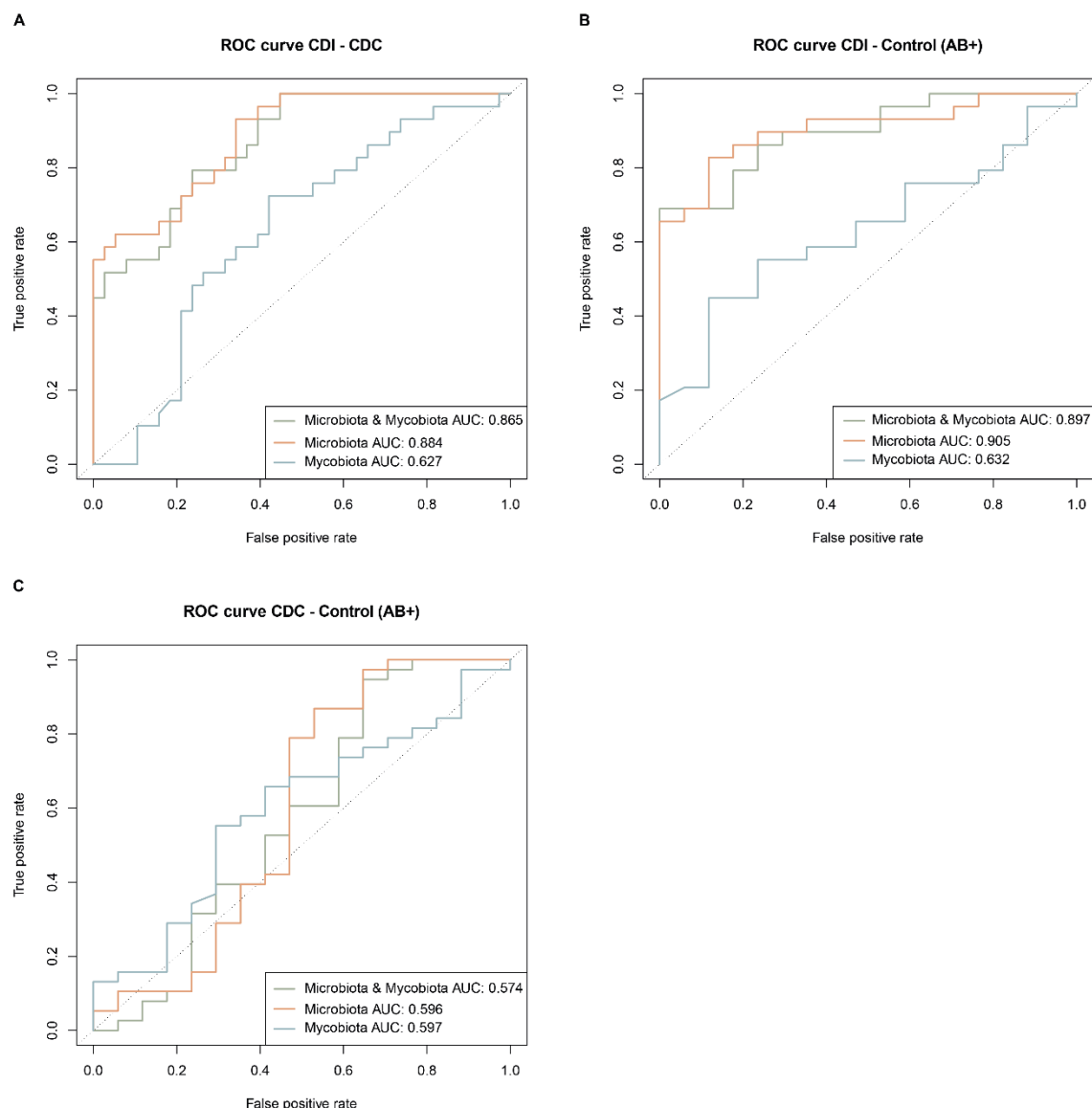


21 **Supplementary Figure 4.** Relative abundance of *Aspergillus* spp. (A) and *Penicillium*
22 spp; (B) in CDI patients, asymptomatic CDC patients and controls (Control). Individual
23 data points are displayed as circles. *** $P \leq 0.001$; ** $P \leq 0.01$ (Wilcoxon Rank Sum
24 Test). CDC: *C. difficile* colonization; CDI: *C. difficile* infection.

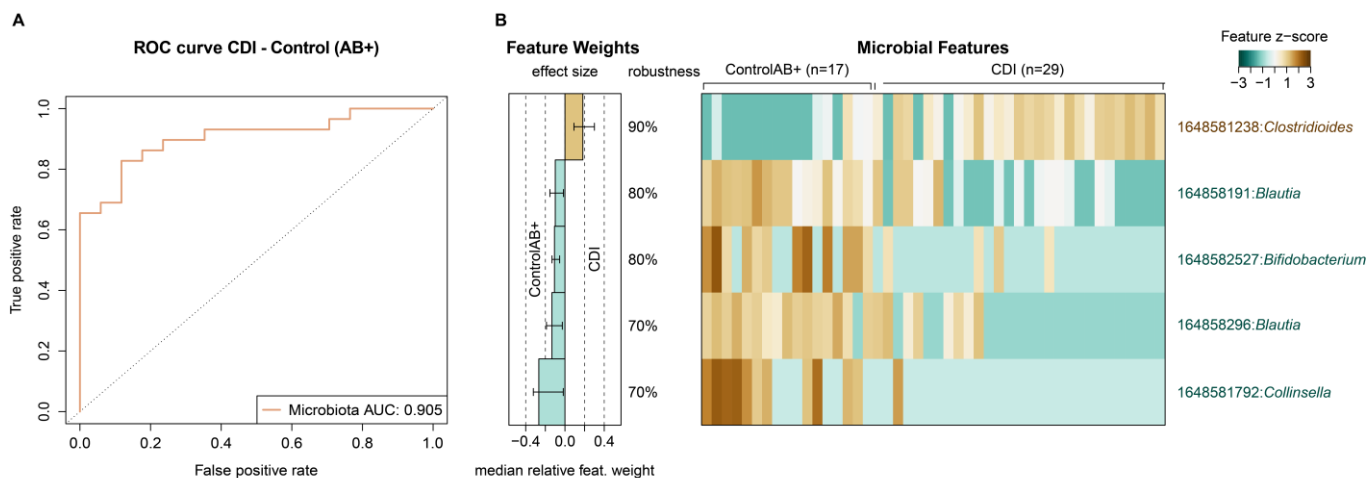


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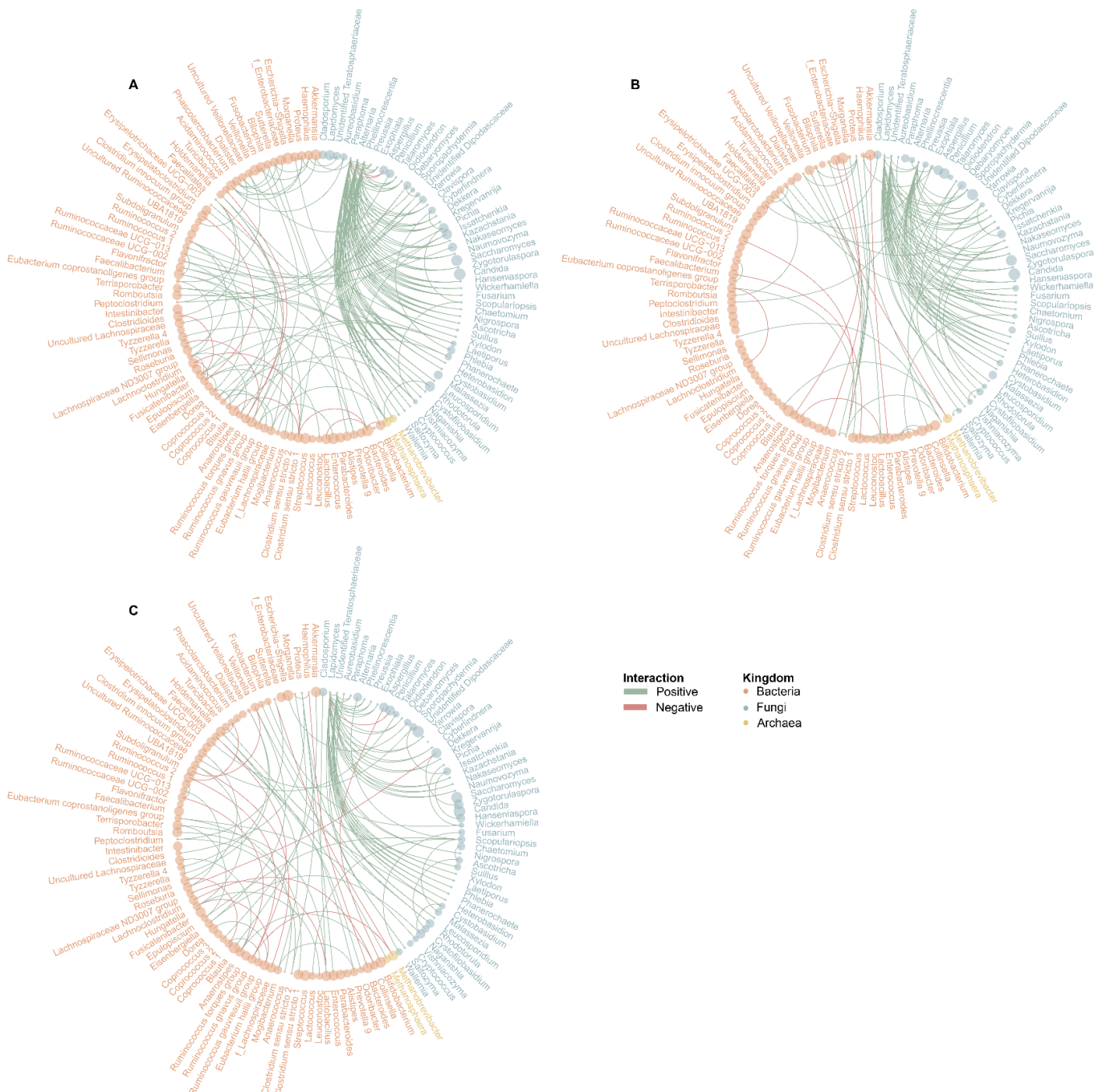
26 **Supplementary Figure 5.** Shannon diversity of the mycobiota in CDI patients,
27 asymptomatic CDC patients and controls (Control). Non-significant results are not
28 displayed. CDC: *C. difficile* colonization; CDI: *C. difficile* infection.



29 **Supplementary Figure 6.** Receiver Operating Characteristic (ROC) curve for the
 30 models distinguishing the groups. ROC curves are displayed for joint microbiota and
 31 mycobiota data (green), microbiota data (orange) and mycobiota data (blue) with
 32 corresponding Area Under the Curves (AUCs). (A) models distinguishing CDI patients
 33 from asymptomatic CDC patients; (B) models distinguishing CDI patients from
 34 controls who received antibiotic treatment (Control AB+); (C) models distinguishing
 35 asymptomatic CDC patients from controls who received antibiotic treatment (Control
 36 AB+). CDC: *C. difficile* colonization; CDI: *C. difficile* infection.



37 **Supplementary Figure 7.** Signature of gut bacterial OTUs associated to CDI patients
 38 and controls who received antibiotic treatment (ControlAB+). (A) Cross-validation
 39 accuracy of the microbiota classifier is shown in the ROC curve with the mean AUC;
 40 (B) Median relative feature weights of the selected gut bacterial OTUs show the
 41 contribution of each marker OTU to the classification. Robustness of the selected
 42 features indicates the fraction of models containing the specific feature. The normalized
 43 values of the selected features across CDI and ControlAB+ is shown in the heatmap.
 44 AUC: Area Under the Curve; CDC: *C. difficile* colonization; CDI: *C. difficile* infection;
 45 ROC: Receiver Operating Characteristic.



46 **Supplementary Figure 8.** Fungal-bacterial networks. Positive interactions are
 47 highlighted in green, while negative interactions are highlighted in red. Kingdoms are
 48 represented by color; mean relative abundance of the genus are represented by point
 49 size. (A) CDI patients; (B) CDC patients; (C) controls who received antibiotic
 50 treatment (ControlAB+). CDC: *C. difficile* colonization; CDI: *C. difficile* infection.

51 **Supplementary Table 1. Subject characteristics of CDI patients, CDC patients and**
 52 **controls (Control)**

	CDI (<i>n</i> = 29)	CDC (<i>n</i> = 38)	Control (<i>n</i> = 38)
Age	59.52 (17.640)	54.89 (19.316)	57.00 (13.981)
Sex			
Male	16 (55.2%)	20 (52.6%)	24 (63.2%)
Female	13 (44.8%)	18 (47.4%)	14 (36.8%)
Hospital			
Amphia	2 (6.9%)	24 (63.2%)	23 (60.5%)
LUMC	27 (93.1%)	14 (36.8%)	15 (39.5%)
Previous CDI			
Last 8 weeks	3 (10.3%)	1 (2.6%)	0 (0.0%)
>8 weeks earlier	2 (6.9%)	3 (7.9%)	0 (0.0%)
Hospitalization (last year)			
IBD	2 (6.9%)	7 (18.4%)	1 (2.6%)
Solid organ transplant	9 (31.0%)	7 (18.4%)	4 (10.5%)
Solid malignancy	5 (17.2%)	6 (15.8%)	11 (28.9%)
Hematological malignancy	5 (17.2%)	0 (0.0%)	2 (5.3%)
Previous medication use (last three months)			
Antibiotics*	28 (96.6%)	23 (60.5%)	17 (44.7%)
Immunosuppressants	19 (65.5%)	16 (42.1%)	11 (28.9%)
PPI or antacids	23 (79.3%)	27 (71.1%)	18 (47.4%)
Chemotherapy	7 (24.1%)	2 (5.3%)	4 (10.5%)

53 CDC: *C. difficile* colonization; CDI: *C. difficile* infection. *Previous antibiotic use
 54 uncertain in six CDC samples (15.8%) and six control samples (15.8%). This table is
 55 adapted with permission from Crobach *et al.* published in *Microorganisms*^[29].