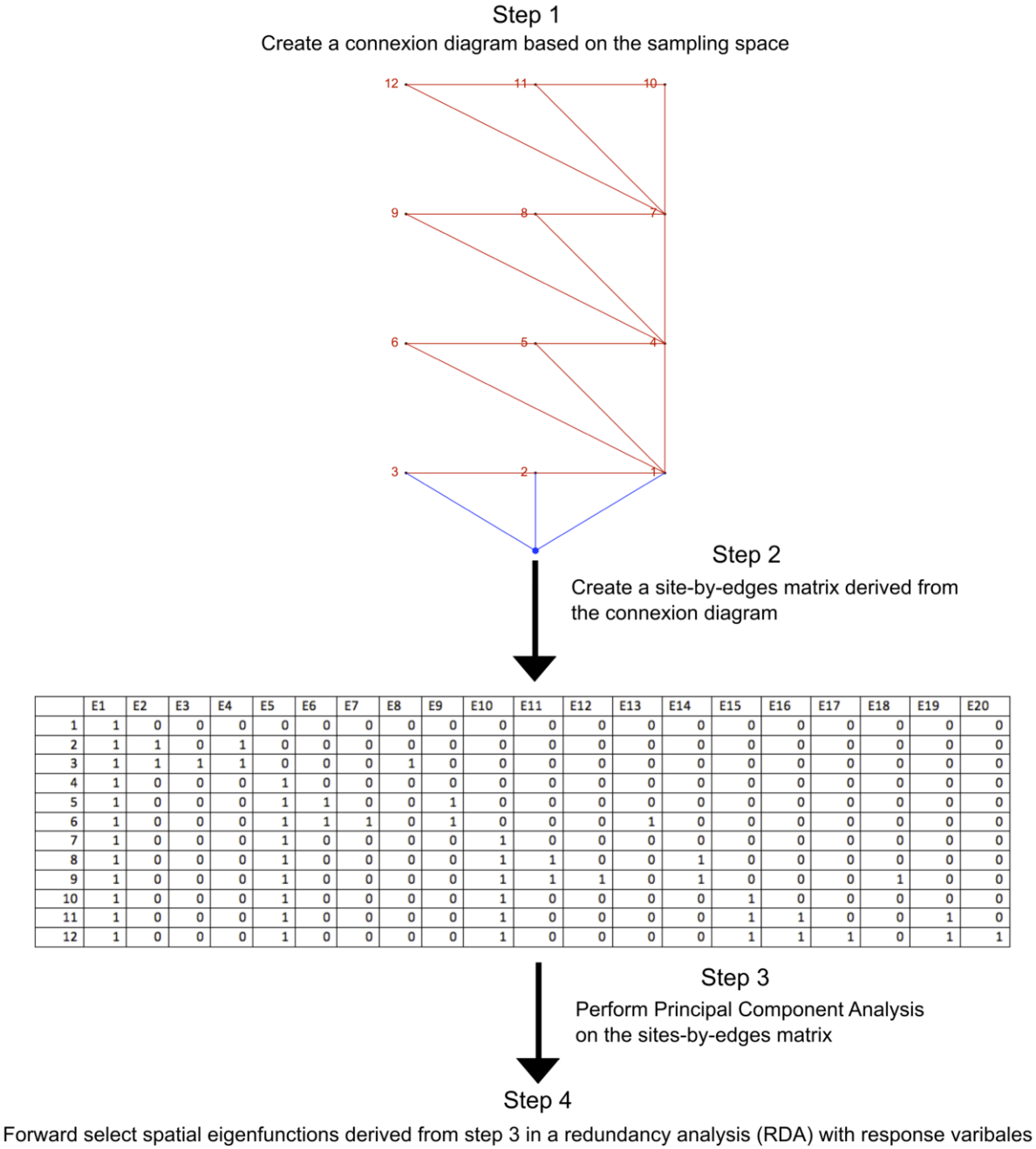
Supplementary figure Legends

Supplementary figure 1

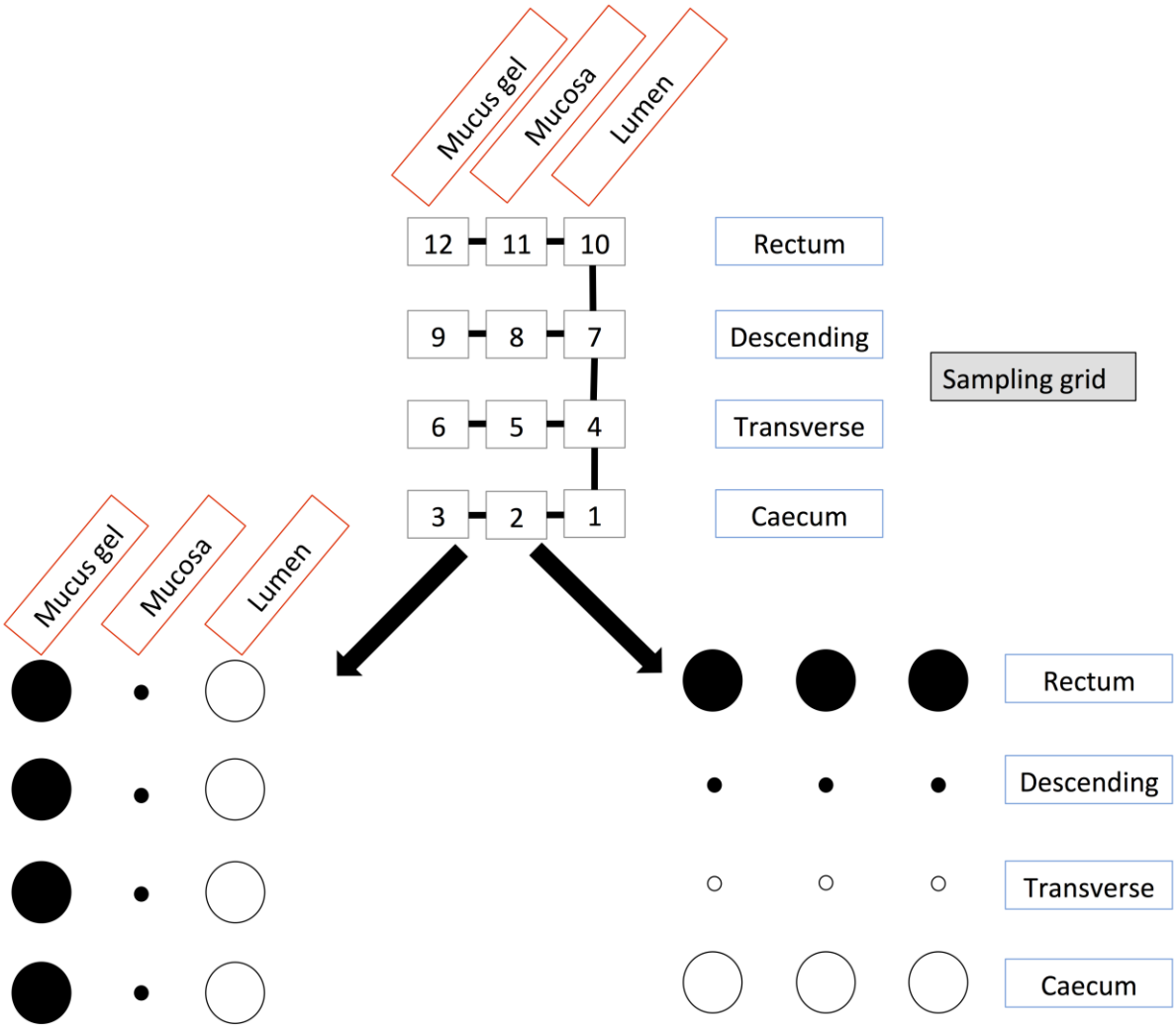
Workflow for generating spatial eigenvectors from the connexion matrix.



	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13	E14	E15	E16	E17	E18	E19	E20
1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	1	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
4	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	1	0	0	0	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0
6	1	0	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	0	0	0
7	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
8	1	0	0	0	1	0	0	0	0	1	1	0	0	1	0	0	0	0	0	0
9	1	0	0	0	1	0	0	0	0	1	1	1	0	1	0	0	0	1	0	0
10	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0
11	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	0	0	1	0
12	1	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	0	1	1

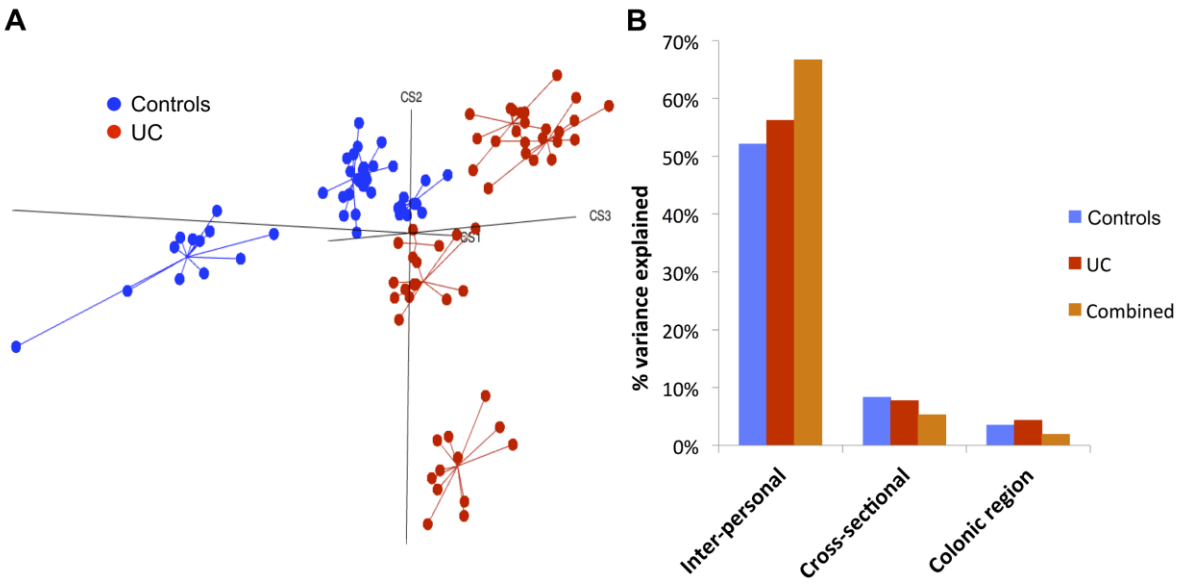
Supplementary figure 2

A diagram illustrating the sampling grid and how idealised cross-sectional and longitudinal gradients are represented by fitted RDA values. This is provided as an aid to interpreting Figure 3.



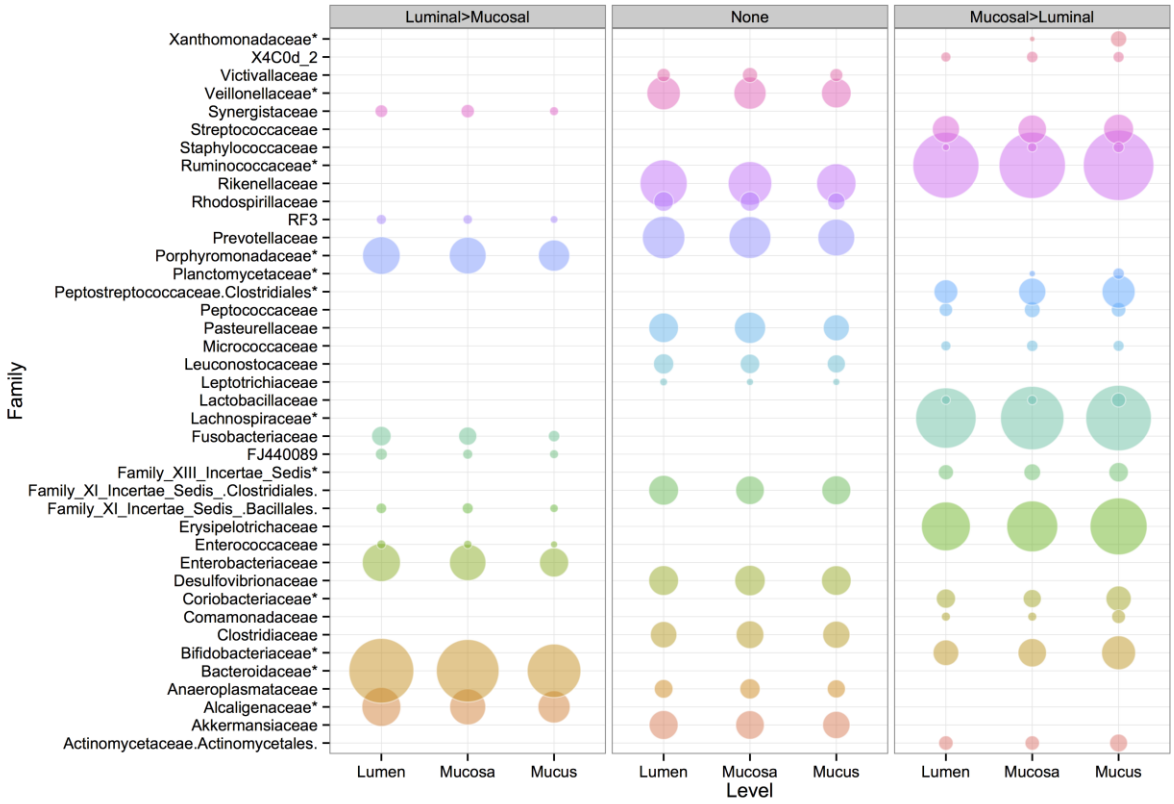
Supplementary figure 3

Between Class Analysis (BCA) of Hellinger-transformed family-level abundances (A). Percentage of variance explained by the BCA (B).



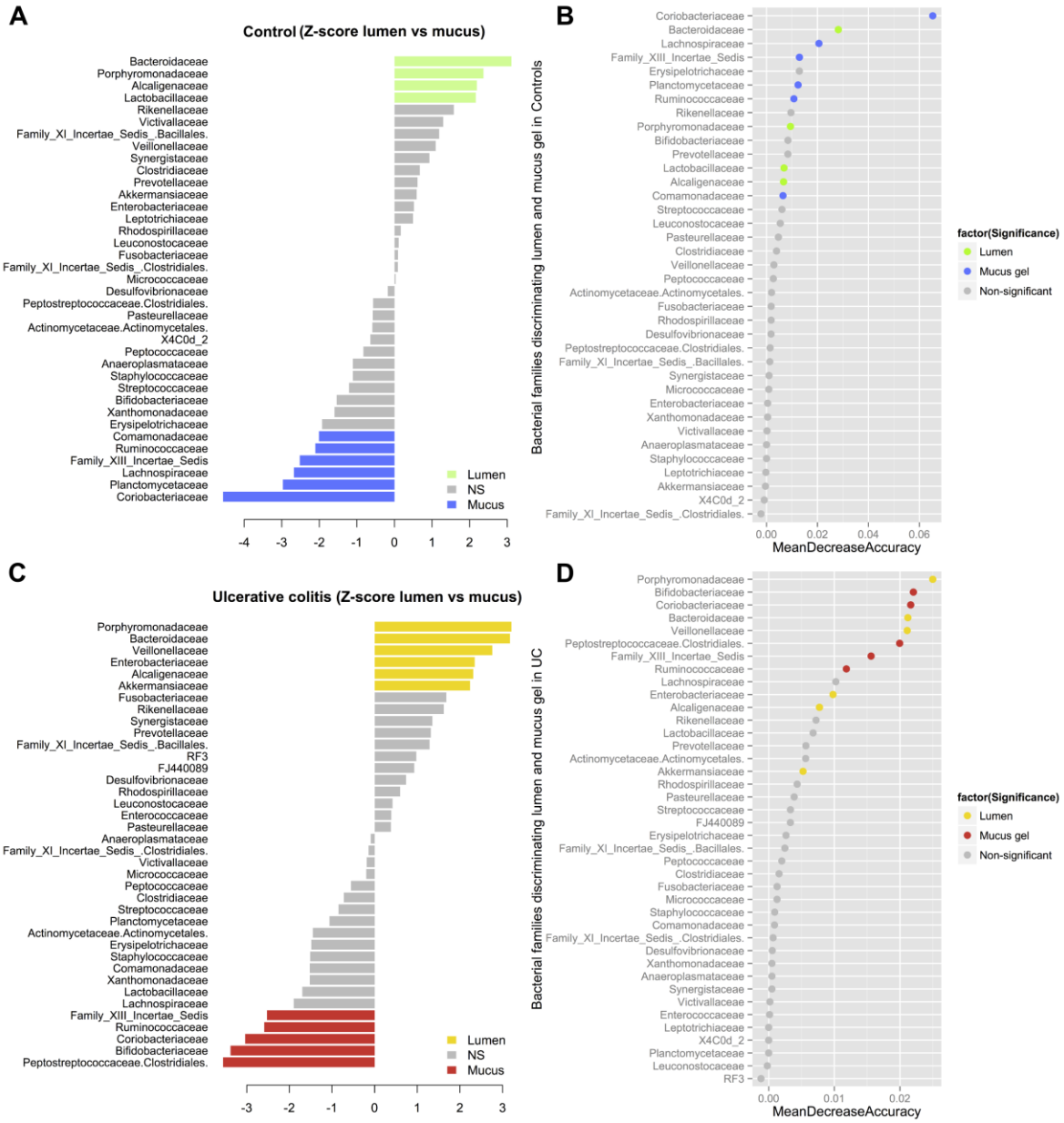
Supplementary figure 4

Bacterial family-level abundances represented from the lumen to mucosa to mucus gel for all samples in both cohorts combined. The families are split into three groups; those that show a greater abundance in luminal compared to the mucosal layers, those that show no gradient and those that are more abundant in the mucosal layers when compared to the lumen. Abundances are represented proportionally to the area by bubble plots and the mean abundances with standard deviation are given in Supplementary table 2. An asterisk is placed beside those families that demonstrate a stasticially significant difference across the three levels as determined by the Kruskal-Wallis test.



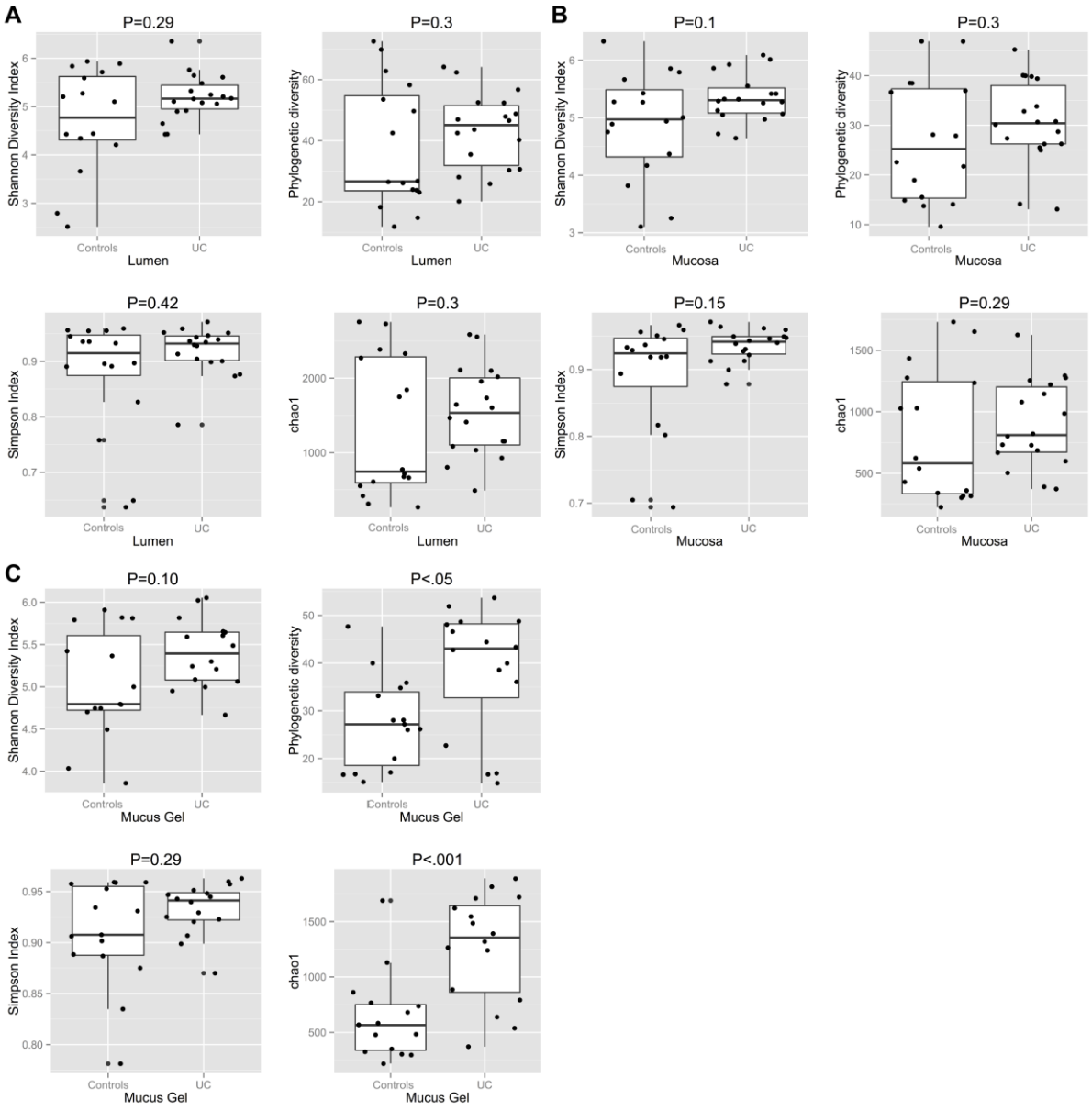
Supplementary figure 5

(A) Z-scores from the Wilcoxon test, ranked from most abundant in the lumen to most abundant in the mucus gel, colored by significance, in controls. (B) Corresponding Random Forests results, color-coded as in A. (C) Z-scores from the Wilcoxon test, ranked from most abundant in the lumen to most abundant in the mucus gel, colored by significance, in UC. (D) Corresponding Random Forests results, color-coded as in C.


A
Control (Z-score lumen vs mucus)
Bacteroidaceae
Porphyromonadaceae
Alcaligenaceae
Lactobacillaceae
Rikenellaceae
Victivallaceae
Family_XI_Incertae_Sedis_.Bacillales.
Veillonellaceae
Synergistaceae
Clostridiaceae
Prevotellaceae
Akkermansiaceae
Enterobacteriaceae
Leptotrichiaceae
Rhodospirillaceae
Leuconostocaceae
Fusobacteriaceae
Family_XI_Incertae_Sedis_.Clostridiales.
Micrococcaceae
Desulfovibrionaceae
Peptostreptococcaceae.Clostridiales.
Pasteurellaceae
Actinomycetaceae.Actinomycetales.
X4C0d_2
Peptococcaceae
Anaeroplasmataceae
Staphylococcaceae
Streptococcaceae
Bifidobacteriaceae
Xanthomonadaceae
Erysipelotrichaceae
Comamonadaceae
Ruminococcaceae
Family_XIII_Incertae_Sedis
Lachnospiraceae
Planctomycetaceae
Coriobacteriaceae
Lumen
NS
Mucus
-4 -3 -2 -1 0 1 2 3
B
Bacterial families discriminating lumen and mucus gel in Controls
Coriobacteriaceae
Bacteroidaceae
Lachnospiraceae
Family_XIII_Incertae_Sedis
Erysipelotrichaceae
Planctomycetaceae
Ruminococcaceae
Rikenellaceae
Porphyromonadaceae
Bifidobacteriaceae
Prevotellaceae
Lactobacillaceae
Alcaligenaceae
Comamonadaceae
Streptococcaceae
Leuconostocaceae
Pasteurellaceae
Clostridiaceae
Veillonellaceae
Peptococcaceae
Actinomycetaceae.Actinomycetales.
Fusobacteriaceae
Rhodospirillaceae
Desulfovibrionaceae
Peptostreptococcaceae.Clostridiales.
Family_XI_Incertae_Sedis_.Bacillales.
Synergistaceae
Micrococcaceae
Enterobacteriaceae
Xanthomonadaceae
Victivallaceae
Anaeroplasmataceae
Staphylococcaceae
Leptotrichiaceae
Akkermansiaceae
X4C0d_2
Family_XI_Incertae_Sedis_.Clostridiales.
0.00 0.02 0.04 0.06
MeanDecreaseAccuracy
factor(Significance)
Lumen
Mucus gel
Non-significant
C
Ulcerative colitis (Z-score lumen vs mucus)
Porphyromonadaceae
Bacteroidaceae
Veillonellaceae
Enterobacteriaceae
Alcaligenaceae
Akkermansiaceae
Fusobacteriaceae
Rikenellaceae
Synergistaceae
Prevotellaceae
Family_XI_Incertae_Sedis_.Bacillales.
RF3
FJ440089
Desulfovibrionaceae
Rhodospirillaceae
Leuconostocaceae
Enterococcaceae
Pasteurellaceae
Anaeroplasmataceae
Family_XI_Incertae_Sedis_.Clostridiales.
Victivallaceae
Micrococcaceae
Peptococcaceae
Clostridiaceae
Streptococcaceae
Planctomycetaceae
Actinomycetaceae.Actinomycetales.
Erysipelotrichaceae
Staphylococcaceae
Comamonadaceae
Xanthomonadaceae
Lactobacillaceae
Lachnospiraceae
Family_XIII_Incertae_Sedis
Ruminococcaceae
Coriobacteriaceae
Bifidobacteriaceae
Peptostreptococcaceae.Clostridiales.
Lumen
NS
Mucus
-3 -2 -1 0 1 2 3
D
Bacterial families discriminating lumen and mucus gel in UC
Porphyromonadaceae
Bifidobacteriaceae
Coriobacteriaceae
Bacteroidaceae
Veillonellaceae
Peptostreptococcaceae.Clostridiales.
Family_XIII_Incertae_Sedis
Ruminococcaceae
Lachnospiraceae
Enterobacteriaceae
Alcaligenaceae
Rikenellaceae
Lactobacillaceae
Prevotellaceae
Actinomycetaceae.Actinomycetales.
Akkermansiaceae
Rhodospirillaceae
Pasteurellaceae
Streptococcaceae
FJ440089
Erysipelotrichaceae
Family_XI_Incertae_Sedis_.Bacillales.
Peptococcaceae
Clostridiaceae
Fusobacteriaceae
Micrococcaceae
Staphylococcaceae
Comamonadaceae
Family_XI_Incertae_Sedis_.Clostridiales.
Desulfovibrionaceae
Xanthomonadaceae
Anaeroplasmataceae
Synergistaceae
Victivallaceae
Enterococcaceae
Leptotrichiaceae
X4C0d_2
Planctomycetaceae
Leuconostocaceae
RF3
0.00 0.01 0.02
MeanDecreaseAccuracy
factor(Significance)
Lumen
Mucus gel
Non-significant

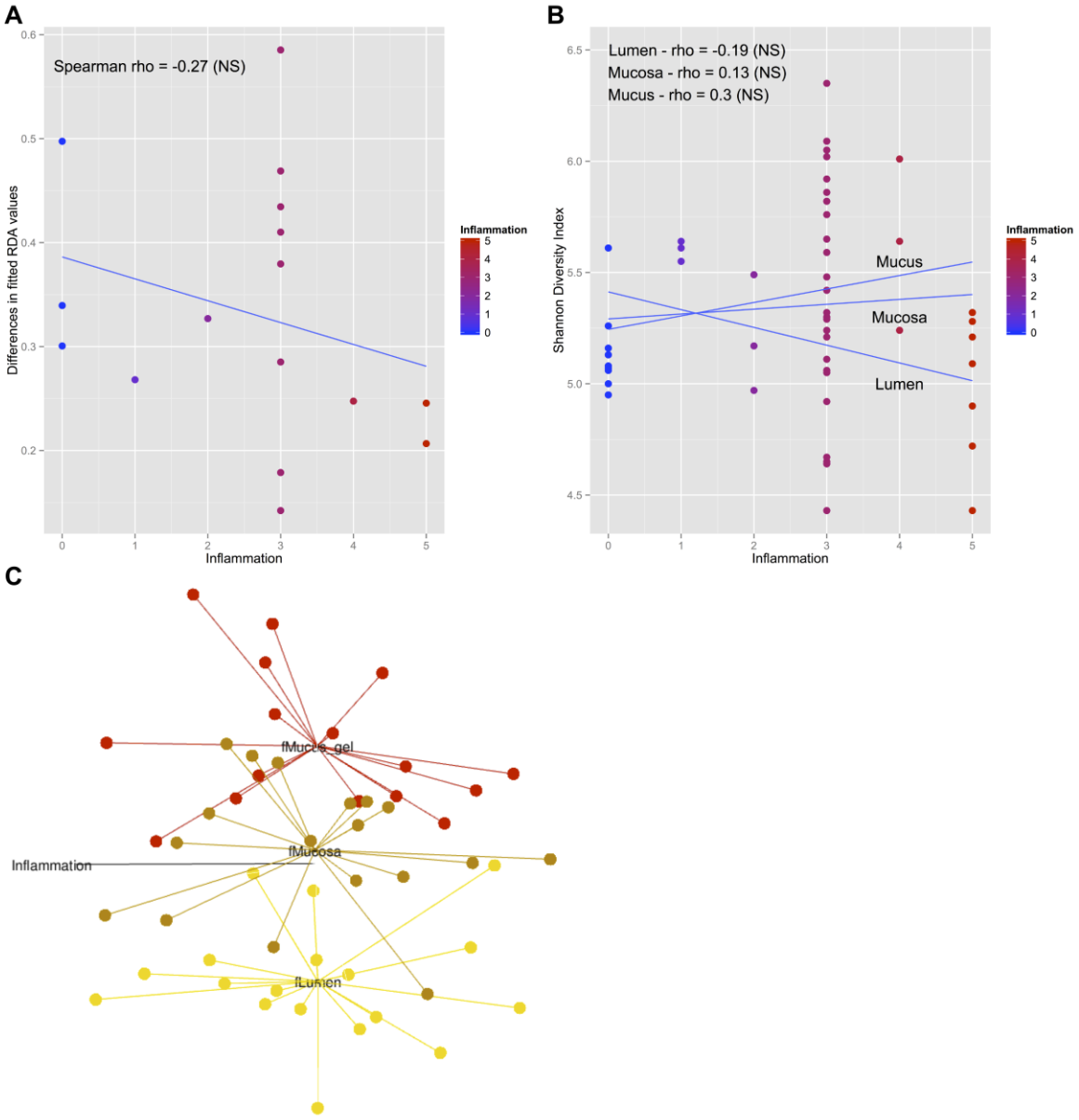
Supplementary figure 6

Boxplots of four alpha diversity indices, Shannon diversity, Simpson index, chao1 and Faith's phylogenetic diversity, for the three cross-sectional spatial samples sets, luminal (A), whole mucosal biopsies (B) and mucus gel layer (C).



Supplementary figure 7

Effects of regional inflammation on the microbiota. Differences in fitted RDA values from the AEM analysis (Figure 3C and 3D) compared with the matched inflammatory score, demonstrating no significant correlation (A). Microbial diversity, as measured by the Shannon Diversity Index (H) for luminal, mucosal and mucus gel samples, demonstrating no correlation between alpha-diversity and local inflammation for any of the three spatial locations (B). There was no significant difference between the luminal (Spearman rho -0.19) and mucus gel (Spearman rho 0.3) trends, when compared by non-parametric ANCOVA. Comparison of the luminal, mucosal and mucus gel microbiota in UC using CAP, factoring out inter-personal variability and colorectal location, demonstrating that inflammation is orthogonal to the axis that separates the three spatial locations and thus does not effect one more than the other (C).


A
Spearman rho = -0.27 (NS)
Differences in fitted RDA values
Inflammation
Inflammation
B
Lumen - rho = -0.19 (NS)
Mucosa - rho = 0.13 (NS)
Mucus - rho = 0.3 (NS)
Shannon Diversity Index
Mucus
Mucosa
Lumen
Inflammation
Inflammation
C
fMucus_gel
fMucosa
Inflammation
fLumen