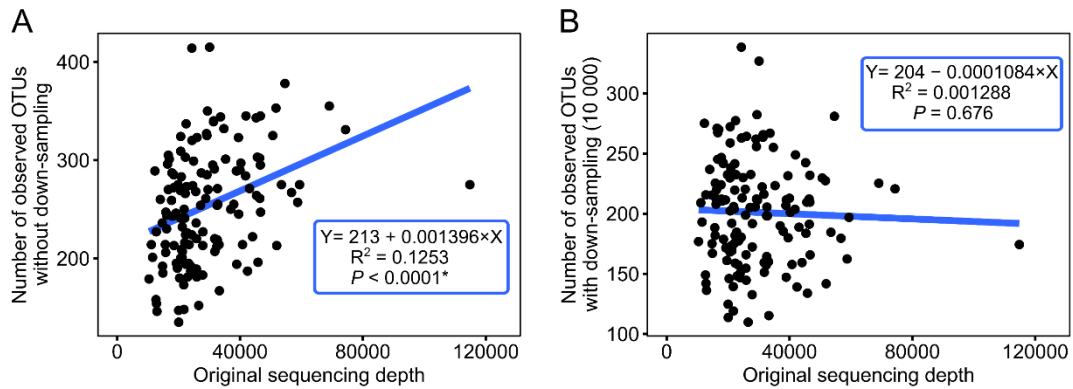Effects of a low FODMAP diet on gut microbiota in individuals with treated celiac disease having persistent gastrointestinal symptoms, Herfindal AM *et al.*

SUPPLEMENTAL FIGURE 1 Relationship between original sequencing depth (10,367 to 114,847 sequences/sample) and number of observed OTUs **(A)** without and **(B)** with down-sampling (rarefying) to an even library size of 10,000 sequences/sample. Each circle represents one sample from one participant ($n = 69$). Blue lines represent the fitted linear regression model with number of observed OTUs as response variable and original sequencing depth as explanatory variable. Blue rectangles contain the model statistics (model estimates (intercept and slope), R^2, and *P* value). Without down-sampling (in A), there is a significant linear relationship between number of observed OTUs and original sequencing depth, which is not present with down-sampling (in B). *: $P < 0.05$.

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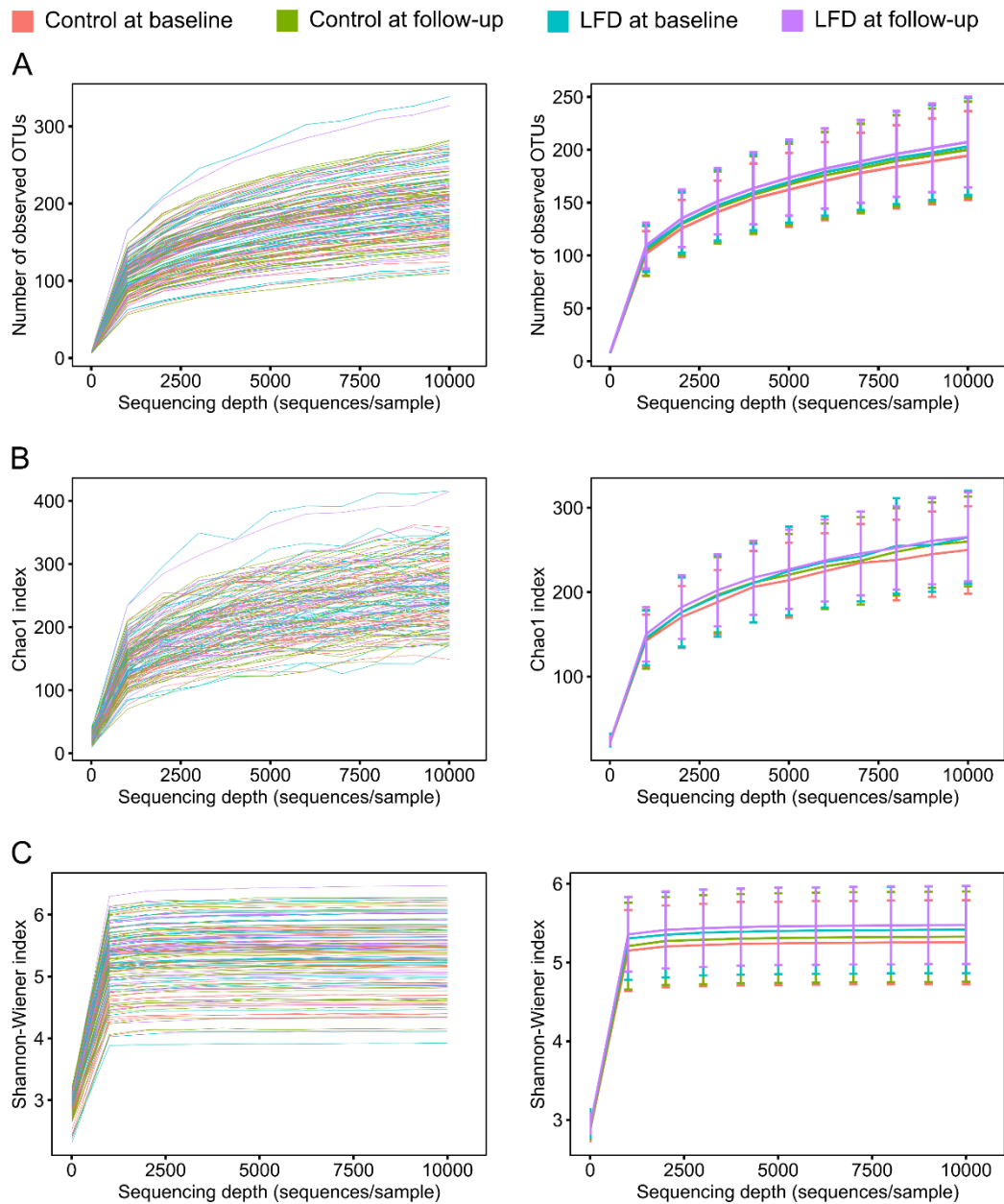

SUPPLEMENTAL FIGURE 2 Rarefaction curves. Relationship between different measures of α-diversity and sequencing depth (sequences/sample) in fecal samples from the control and low FODMAP diet (LFD) group at baseline and 4-week follow-up. **(A)** Number of observed OTUs, **(B)** the Chao1 index, and **(C)** the Shannon-Wiener index of α-diversity. Left panels show the relationship for individual samples (each line represents one sample from one participant) and right panels show group mean ± SD for each diet group-timepoint combination for each sequencing depth (10, 1009, 2008, 3007, 4006, 5005, 6004, 7003, 8002, 9001, and 10 000 sequences/sample). The α-diversity for one sample at one sequencing depth is the mean α-diversity from ten rarefying iterations. FODMAP; fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; OTU, operational taxonomic unit.

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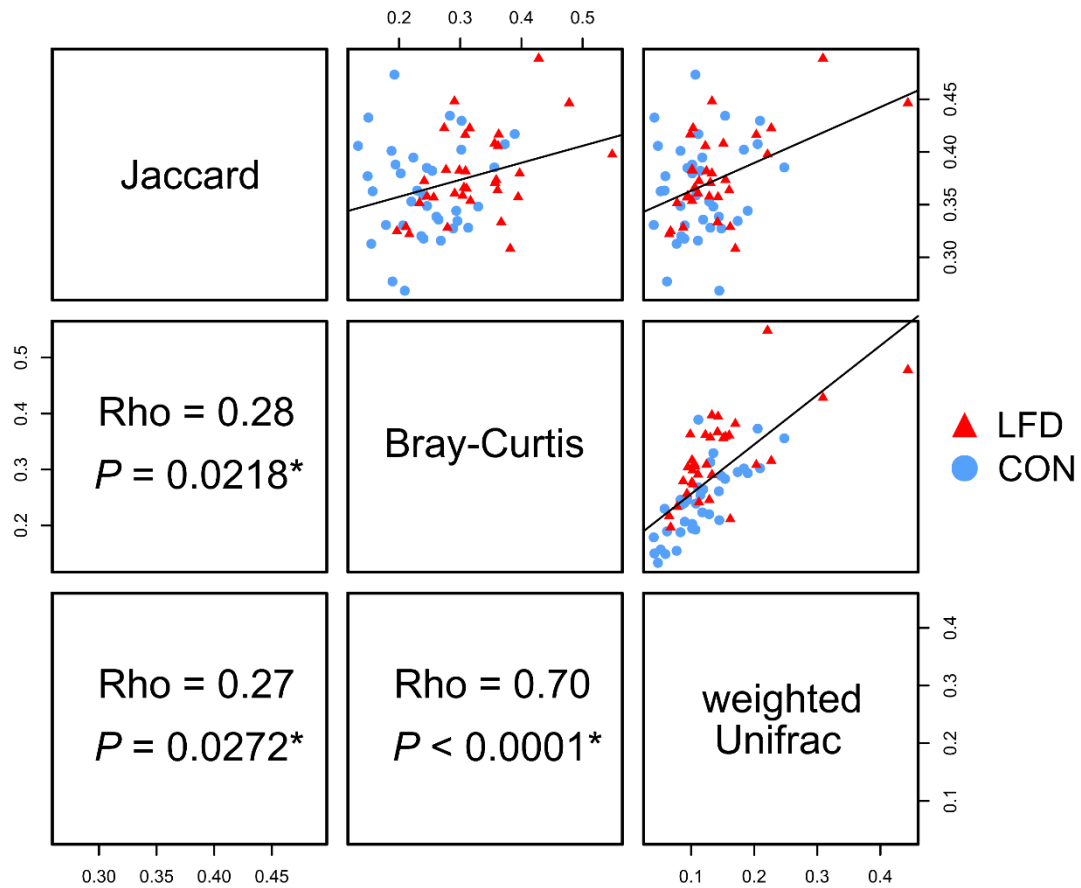

SUPPLEMENTAL FIGURE 3 Relationship between the three measures of within-subject β-diversity (Jaccard, Bray-Curtis, and weighted Unifrac). Panels above the diagonal show scatter plots together with linear regression line where each circle/triangle represents the β-diversity between the baseline and 4-week follow-up sample for one participant in the control (CON, *n* = 36) or low FODMAP diet (LFD, *n* = 33) group. Panels below the diagonal show Spearman's rank correlation coefficients (Rho) together with corresponding *P* values. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. *: *P* < 0.05.

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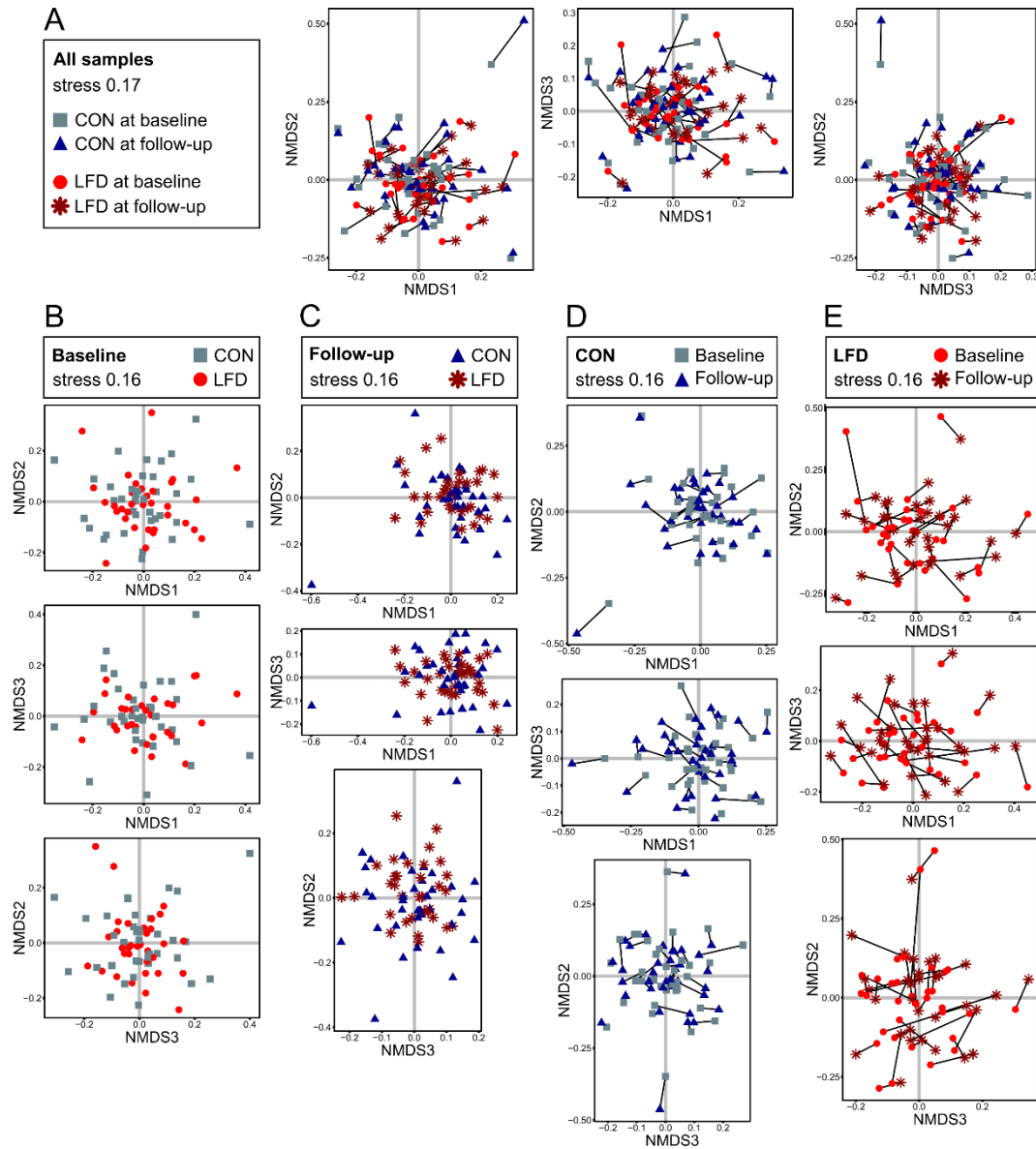

SUPPLEMENTAL FIGURE 4 Ordination plots from non-metric multidimensional scaling (NMDS) of Bray-Curtis distances between samples from **(A)** all participants at both timepoints, **(B)** baseline only, **(C)** 4-week follow-up only, **(D)** participants in the control (CON) group only and **(E)** participants in the low FODMAP diet (LFD) group only. NMDS was performed in three dimensions (NMDS axis 1 vs axis 2, axis 1 vs. axis 3, axis 3 vs. axis 2) and plots were manually inspected in 3D to ensure the representativeness of the presented 2D plots. Three dimensions were chosen because with only two dimensions, convergence problem arose with the metaMDS() function in R. Each symbol represents one sample from one participant, and samples from the same participant are connected with a line. Stress values indicate the NMDS goodness-of-fit. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols.

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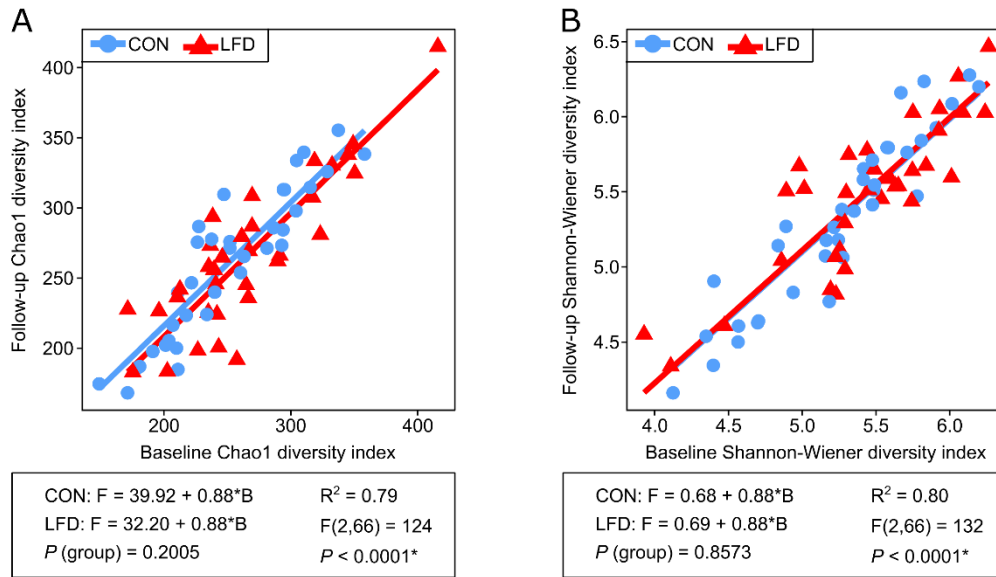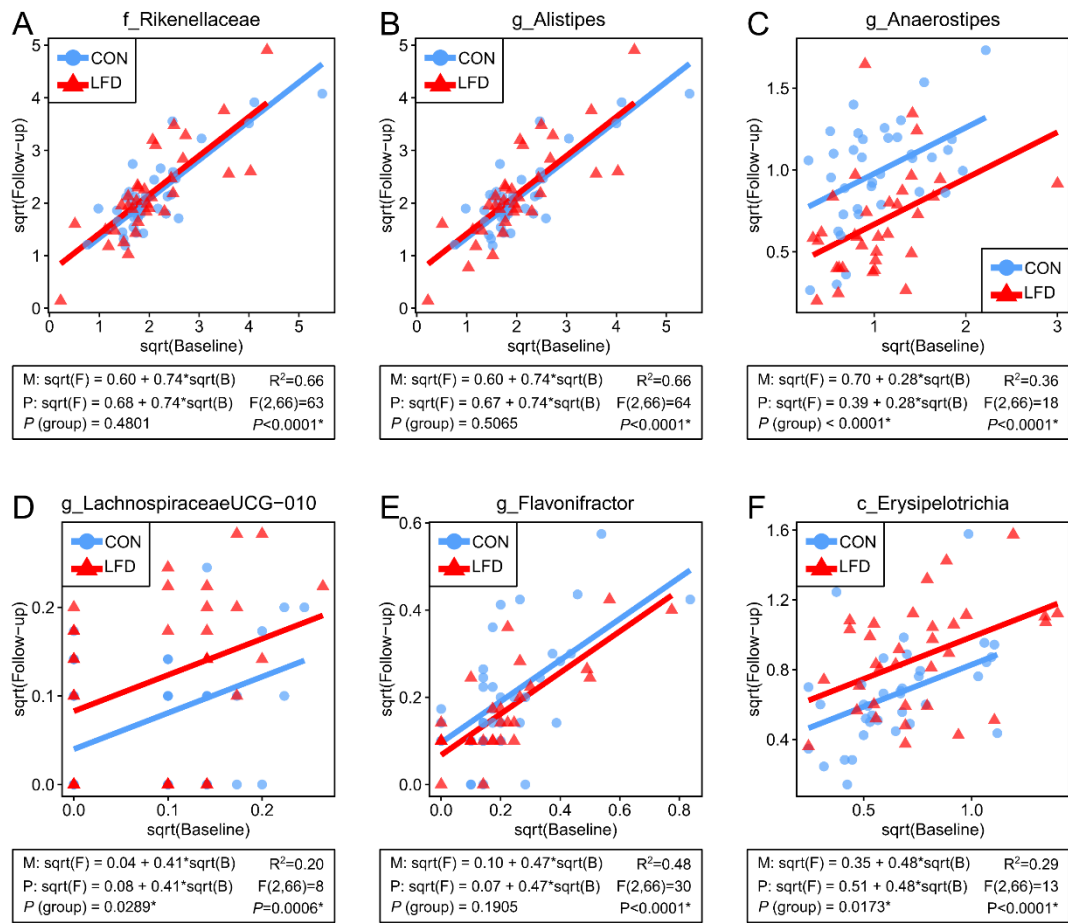

SUPPLEMENTAL FIGURE 5 ANCOVA model plots for α-diversity. Fecal **(A)** Chao1 and **(B)** Shannon-Wiener index, two measures of α-diversity, at baseline and 4-week follow-up for participants in the control (CON, $n = 36$) and low FODMAP diet (LFD, $n = 33$) group. Each circle/triangle represents one participant. Red and blue line represents the fitted linear regression model with 4-week follow-up values as response variable and with baseline values and diet group as explanatory variables. Estimated model equation for each diet group is presented below the graph together with P value for the diet group estimate (difference in intercept between CON and LFD). Statistics for the overall model are also reported (R^2, F(degrees of freedom), and P value). FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. *: $P < 0.05$.

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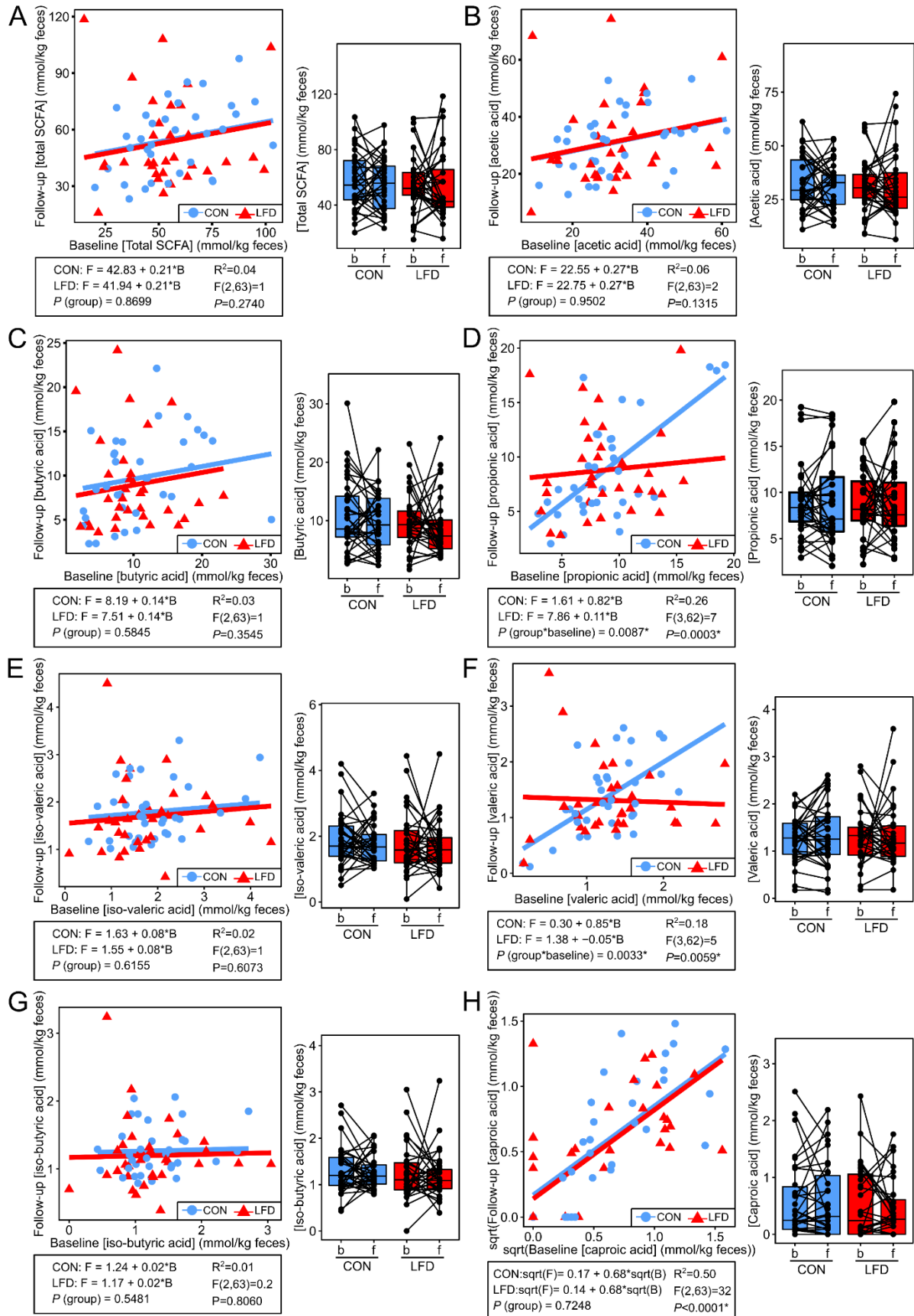

SUPPLEMENTAL FIGURE 6 ANCOVA model plots for bacterial abundances. Fecal relative abundance (%) of (**A**) family *Rikenellaceae*, (**B**) genus *Alistipes*, (**C**) genus *Anaerostipes*, (**D**) genus *Lachnospiraceae UCG-010*, (**E**) genus *Flavonifractor*, and (**F**) class Erysipelotrichia (completely dominated by family *Erysipelotrichaceae*) at baseline and 4-week follow-up for participants in the control (CON, $n = 36$) and low FODMAP diet (LFD, $n = 33$) group. Each circle/triangle represents one participant. Red and blue line representes the fitted linear regression model with follow-up values as response variable and with baseline values and diet group as explanatory variables. Estimated model equation for each diet group is presented below the graph together with P value for the diet group estimate (difference in intercept between CON and LFD). Statistics for the overall model are also reported (R^2, F(degrees of freedom), and P value). FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. *: $P < 0.05$.

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A

CON: F = 42.83 + 0.21*B R^2=0.04
LFD: F = 41.94 + 0.21*B $F_{(2,63)}$=1
P (group) = 0.8699   *P*=0.2740

B

CON: F = 22.55 + 0.27*B R^2=0.06
LFD: F = 22.75 + 0.27*B $F_{(2,63)}$=2
P (group) = 0.9502   *P*=0.1315

C

CON: F = 8.19 + 0.14*B R^2=0.03
LFD: F = 7.51 + 0.14*B $F_{(2,63)}$=1
P (group) = 0.5845   *P*=0.3545

D

CON: F = 1.61 + 0.82*B R^2=0.26
LFD: F = 7.86 + 0.11*B $F_{(3,62)}$=7
P (group*baseline) = 0.0087*   *P*=0.0003*

E

CON: F = 1.63 + 0.08*B R^2=0.02
LFD: F = 1.55 + 0.08*B $F_{(2,63)}$=1
P (group) = 0.6155   P=0.6073

F

CON: F = 0.30 + 0.85*B R^2=0.18
LFD: F = 1.38 + −0.05*B $F_{(3,62)}$=5
P (group*baseline) = 0.0033*   *P*=0.0059*

G

CON: F = 1.24 + 0.02*B R^2=0.01
LFD: F = 1.17 + 0.02*B $F_{(2,63)}$=0.2
P (group) = 0.5481   P=0.8060

H

CON:sqrt(F)= 0.17 + 0.68*sqrt(B) R^2=0.50
LFD:sqrt(F)= 0.14 + 0.68*sqrt(B) $F_{(2,63)}$=32
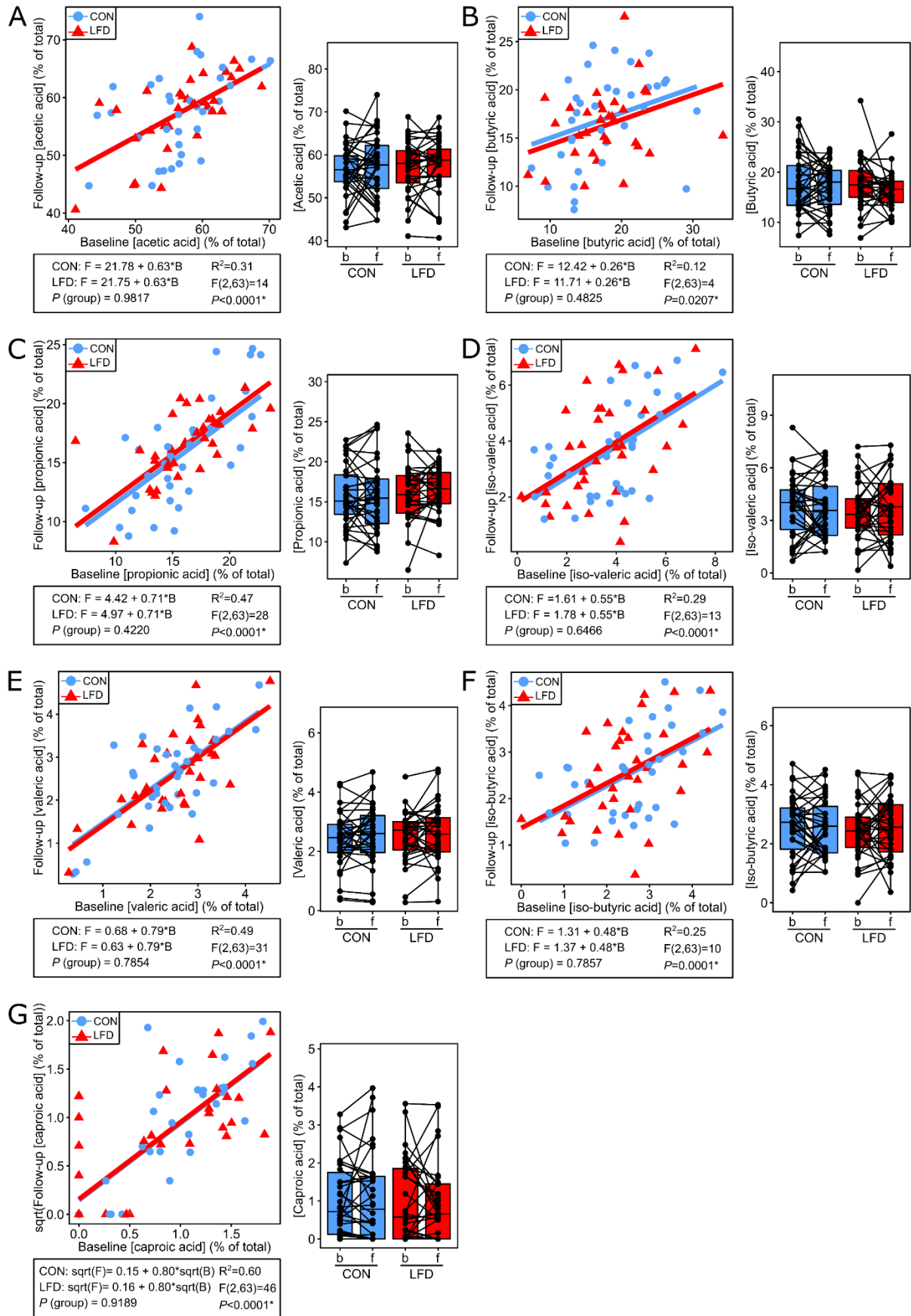*P* (group) = 0.7248   *P*<0.0001*

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SUPPLEMENTAL FIGURE 7 ANCOVA model plots (left panels) and boxplots with individual responses (right panels) for SCFAs. Fecal concentrations (mmol/kg feces) of **(A)** total SCFAs, **(B)** acetic acid, **(C)** butyric acid, **(D)** propionic acid, **(E)** iso-valeric acid, **(F)** valeric acid, **(G)** iso-butyric acid, and **(H)** caproic acid at baseline (b) and 4-week follow-up (f) for the control (CON, $n = 34$) and low FODMAP diet (LFD, $n = 32$) group. In left panels, each circle/triangle represents one participant. Red and blue line representes the fitted linear regression model with follow-up values as response variable and with baseline values and diet group as explanatory variables. Estimated model equation for each diet group is presented below the graph together with P value for the diet group (difference in intercept between CON and LFD) or interaction estimate (difference in slope between CON and LFD). Statistics for the overall model are also reported (R^2, F(degrees of freedom), and P value). In right panels, each circle represents one sample from one participant, and samples from the same participant are connected with a line. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. *: $P < 0.05$.

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A

CON: F = 21.78 + 0.63*B R^2=0.31
LFD: F = 21.75 + 0.63*B   F(2,63)=14
P (group) = 0.9817   *P*<0.0001*

B

CON: F = 12.42 + 0.26*B R^2=0.12
LFD: F = 11.71 + 0.26*B   F(2,63)=4
P (group) = 0.4825   *P*=0.0207*

C

CON: F = 4.42 + 0.71*B R^2=0.47
LFD: F = 4.97 + 0.71*B   F(2,63)=28
P (group) = 0.4220   *P*<0.0001*

D

CON: F =1.61 + 0.55*B R^2=0.29
LFD: F = 1.78 + 0.55*B   F(2,63)=13
P (group) = 0.6466   *P*<0.0001*

E

CON: F = 0.68 + 0.79*B R^2=0.49
LFD: F = 0.63 + 0.79*B   F(2,63)=31
P (group) = 0.7854   *P*<0.0001*

F

CON: F = 1.31 + 0.48*B R^2=0.25
LFD: F = 1.37 + 0.48*B   F(2,63)=10
P (group) = 0.7857   *P*=0.0001*

G

CON: sqrt(F)= 0.15 + 0.80*sqrt(B) R^2=0.60
LFD: sqrt(F)= 0.16 + 0.80*sqrt(B)   F(2,63)=46
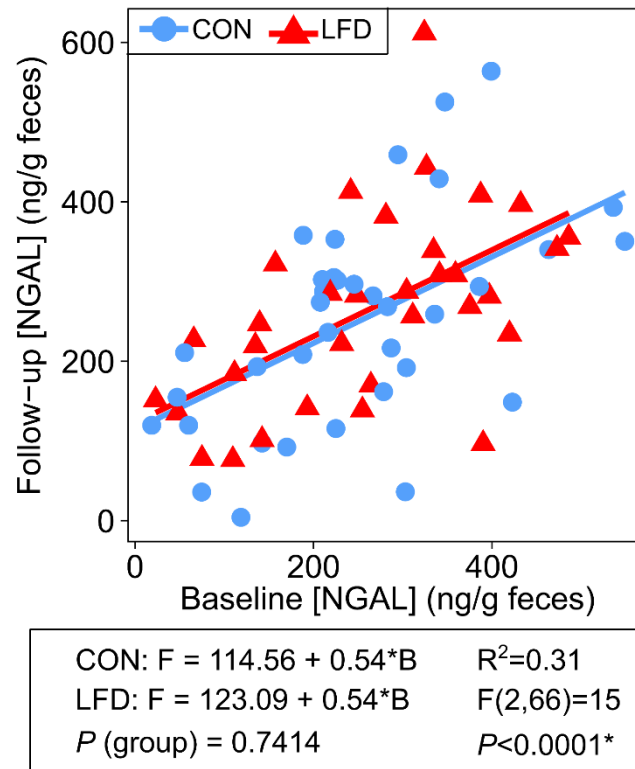*P* (group) = 0.9189   *P*<0.0001*

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SUPPLEMENTAL FIGURE 8 ANCOVA model plots (left panels) and boxplots with individual responses (right panels) for SCFAs. Fecal relative amounts (% of total) of **(A)** acetic acid, **(B)** butyric acid, **(C)** propionic acid, **(D)** iso-valeric acid, **(E)** valeric acid, **(F)** iso-butyric acid, and **(G)** caproic acid at baseline (b) and 4-week follow-up (f) for the control (CON, $n = 34$) and low FODMAP diet (LFD, $n = 32$) group. In left panels, each circle/triangle represents one participant. Red and blue line representes the fitted linear regression model with follow-up values as response variable and with baseline values and diet group as explanatory variables. Estimated model equation for each diet group is presented below the graph together with P value for the diet group estimate (difference in intercept between CON and LFD). Statistics for the overall model are also reported (R^2, F(degrees of freedom), and P value). In right panels, each circle represents one sample from one participant, and samples from the same participant are connected with a line. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols. *: $P < 0.05$.

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CON: F = 114.56 + 0.54*B R^2=0.31
LFD: F = 123.09 + 0.54*B   F(2,66)=15
P (group) = 0.7414   P<0.0001*

SUPPLEMENTAL FIGURE 9 ANCOVA plot for NGAL. Fecal NGAL concentrations (ng/g feces) at baseline and 4-week follow-up for participants in the control (CON, *n* = 36) and low FODMAP diet (LFD, *n* = 33) group. Each circle/triangle represents one participant. Each circle/triangle represents one participant. Red and blue line representes the fitted linear regression model with follow-up values as response variable and with baseline values and diet group as explanatory variables. Estimated model equation for each diet group is presented below the graph together with *P* value for the diet group estimate (difference in intercept between CON and LFD). Statistics for the overall model are also reported (R^2, F(degrees of freedom), and *P* value). FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; NGAL, neutrophile gelatinase-associated lipocalin. *: *P* < 0.05.

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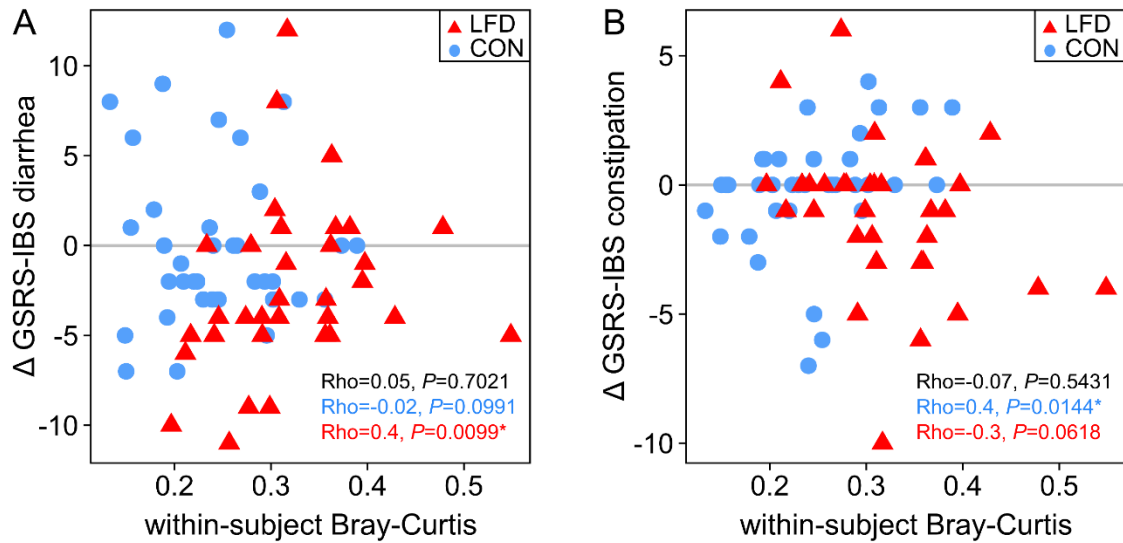
SUPPLEMENTAL FIGURE 10 Relationship between within-subject Bray-Curtis distances and changes in GSRS-IBS **(A)** diarrhea and **(B)** constipation scores. Spearman's rank correlation coefficients (Rho) with corresponding *P* values are presented for the total population (*n* = 69) and separately for the control (CON, *n* = 36) and low FODMAP diet (LFD, *n* = 33) group. Change is defined as 4-week follow-up minus baseline value. Each circle/triangle represents one participant. Within-subject Bray-Curtis distance is defined as the Bray-Curtis distance of the baseline and follow-up sample from the same participant. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; GSRS-IBS, Gastrointestinal Symptom Rating Scale IBS version; IBS, irritable bowel syndrome. *: *P* < 0.05.

Effects of a low FODMAP diet on gut microbiota in individuals with treated celiac disease having persistent gastrointestinal symptoms, Herfindal AM *et al.*
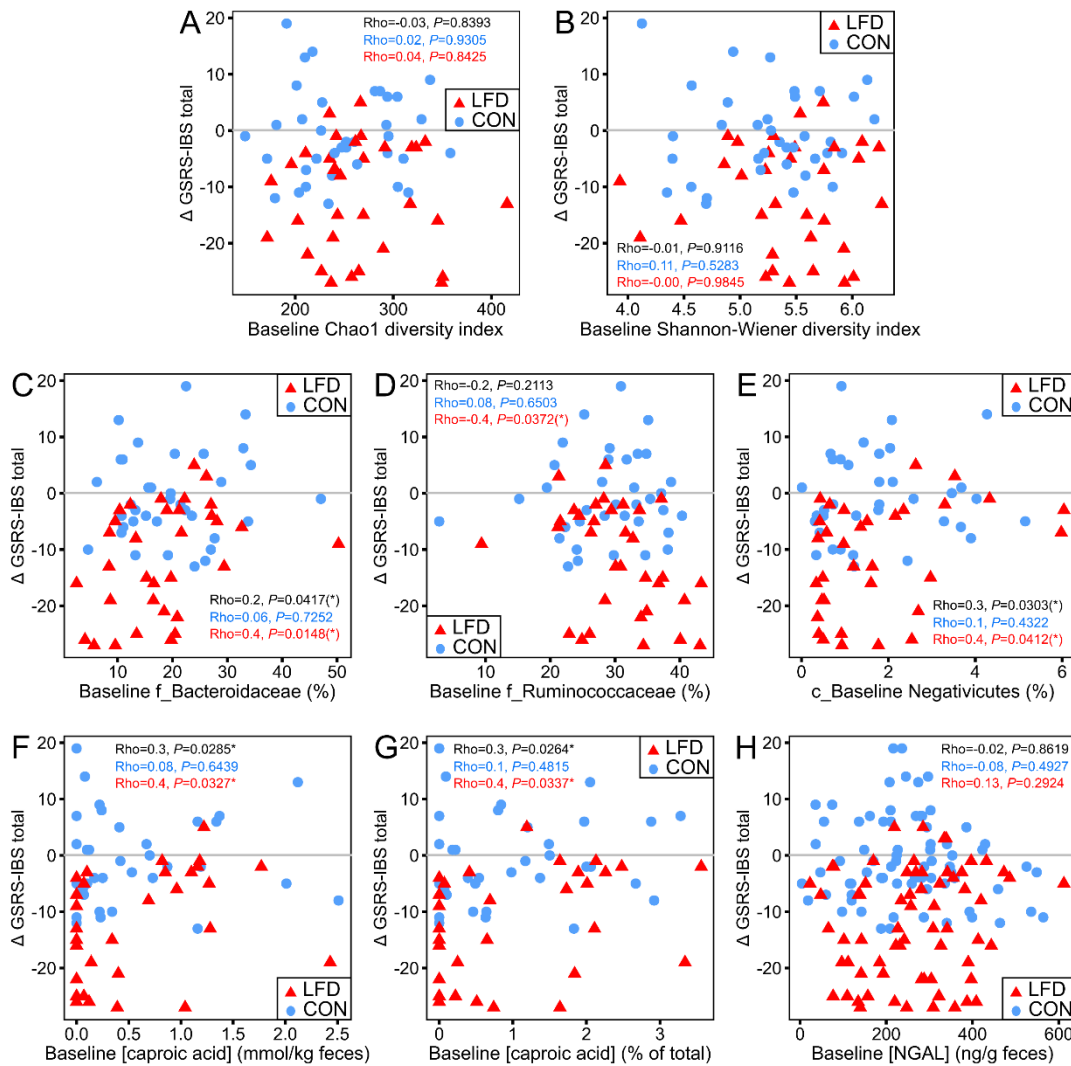
SUPPLEMENTAL FIGURE 11 Relationship between changes in GSRS-IBS total scores and fecal characteristics at baseline. **(A)** Chao1 and **(B)** Shannon-Wiener index of α-diversity. Relative abundance (%) of **(C)** family *Bacteroidaceae* (completely dominated by genus *Bacteroides*), **(D)** family *Ruminococcaceae*, and **(E)** class Negativicutes. Caproic acid **(F)** concentration (mmol/kg feces) and **(G)** relative amounts (% of total). **(H)** NGAL (ng/g feces). Spearman's rank correlation coefficients (Rho) with corresponding *P* values are presented for the total population (*n* = 66-69) and separately for the control (CON, *n* = 34-36) and low FODMAP diet (LFD, *n* = 32-33) group. Change is defined as 4-week follow-up minus baseline value. Each circle/triangle represents one participant. (C-E) No correlations were significant after Benjamini-Hochberg (BH) adjustment of *P* values (performed separately for each taxonomic level). BH adjustments were not performed for A, B, F, G or H. FODMAP, fermentable oligosaccharides, disaccharides, monosaccharides, and polyols; GSRS-IBS, Gastrointestinal Symptom Rating Scale IBS version; IBS, irritable bowel syndrome; NGAL, neutrophile gelatinase-associated lipocalin. (*): unadjusted *P* < 0.05, BH-adjusted *P* > 0.05; *: *P* < 0.05.