

Supplementary Materials for

Gut microbiome responds compositionally and functionally to the seasonal diet variations in wild gibbons

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Lan¹, Xin-Feng Zhao⁴, Li-Nan Huang^{1*}, Peng-Fei Fan^{1*}

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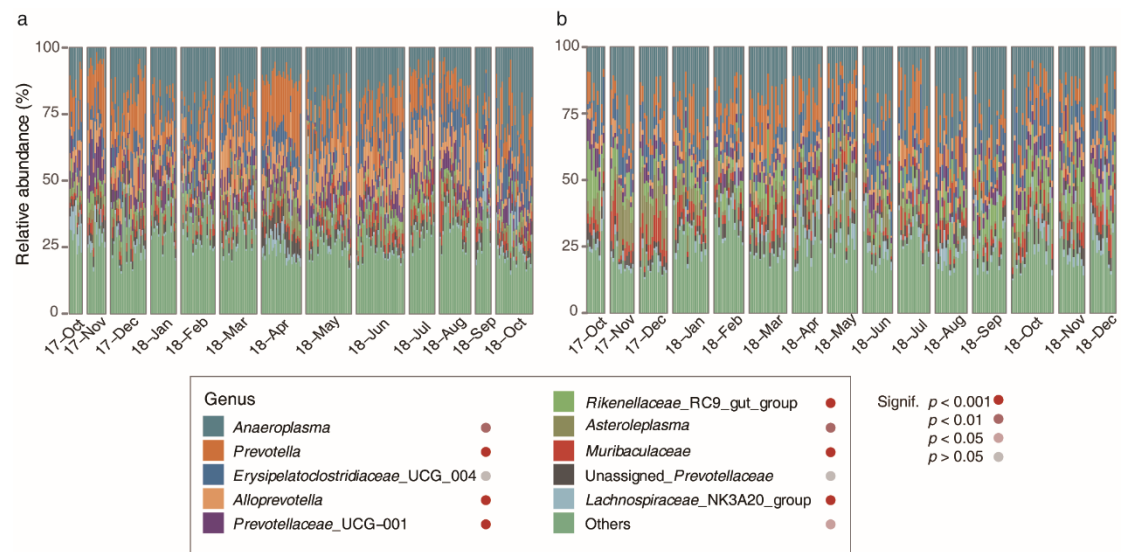
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This PDF file includes:

Supplementary Figure 1 to 13

Other Supplementary Materials for this manuscript include the following:

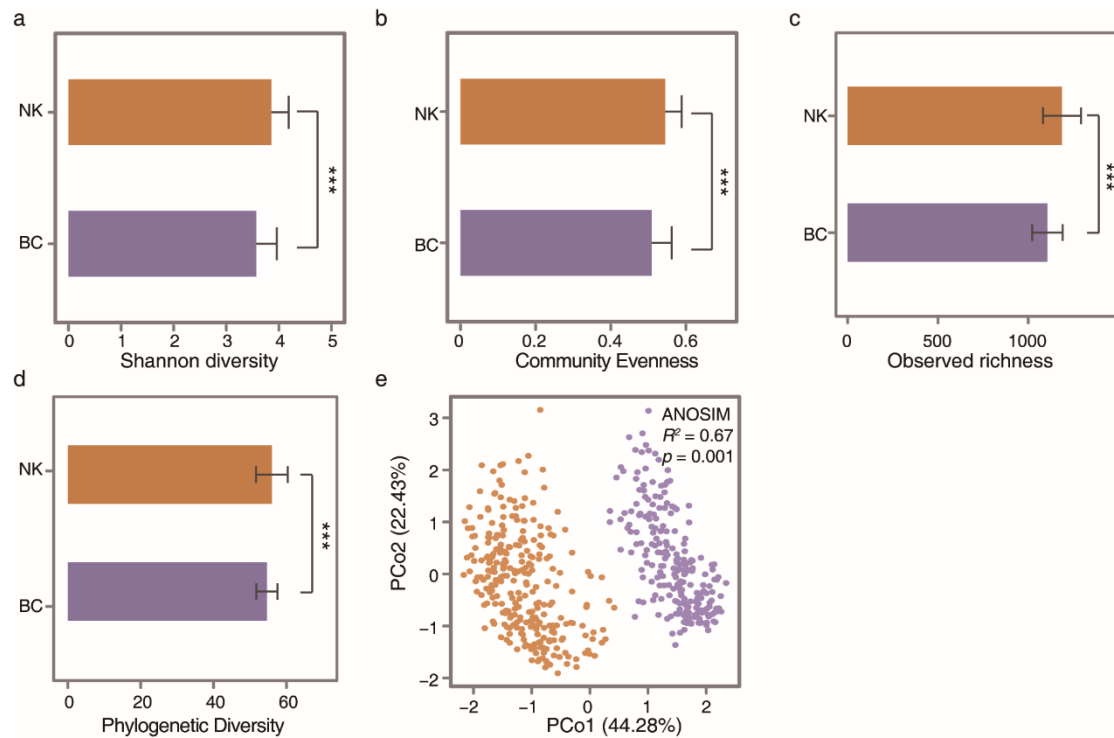
Supplementary Table 1 to 10



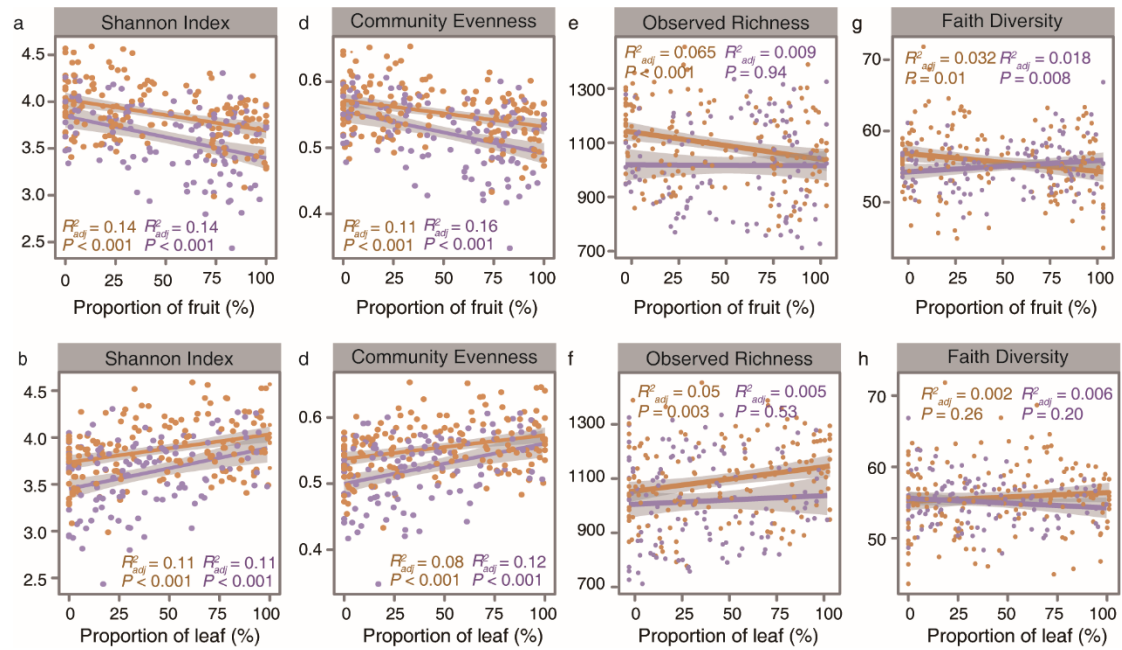
Supplementary Figure 1 | Relative abundances of genera in each fecal microbiome.

Samples were sorted by the sampling time (**a**, NK; **b**, BC). Each column represented one sample and samples from different months was separated by the blank space.

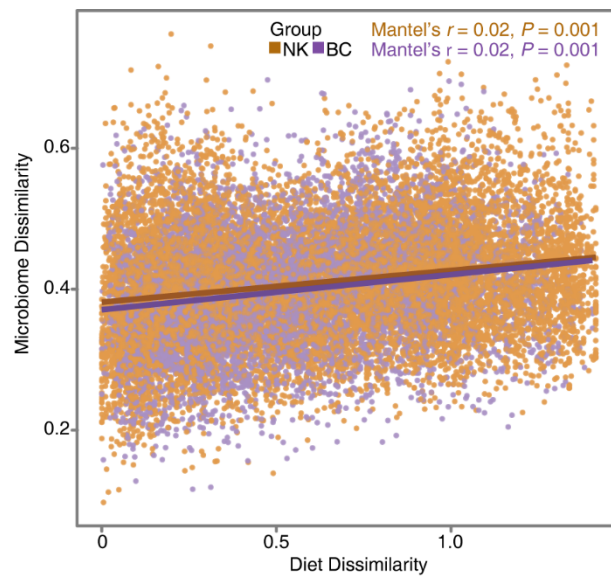
Significance was calculated by the Wilcoxon rank-sum test.



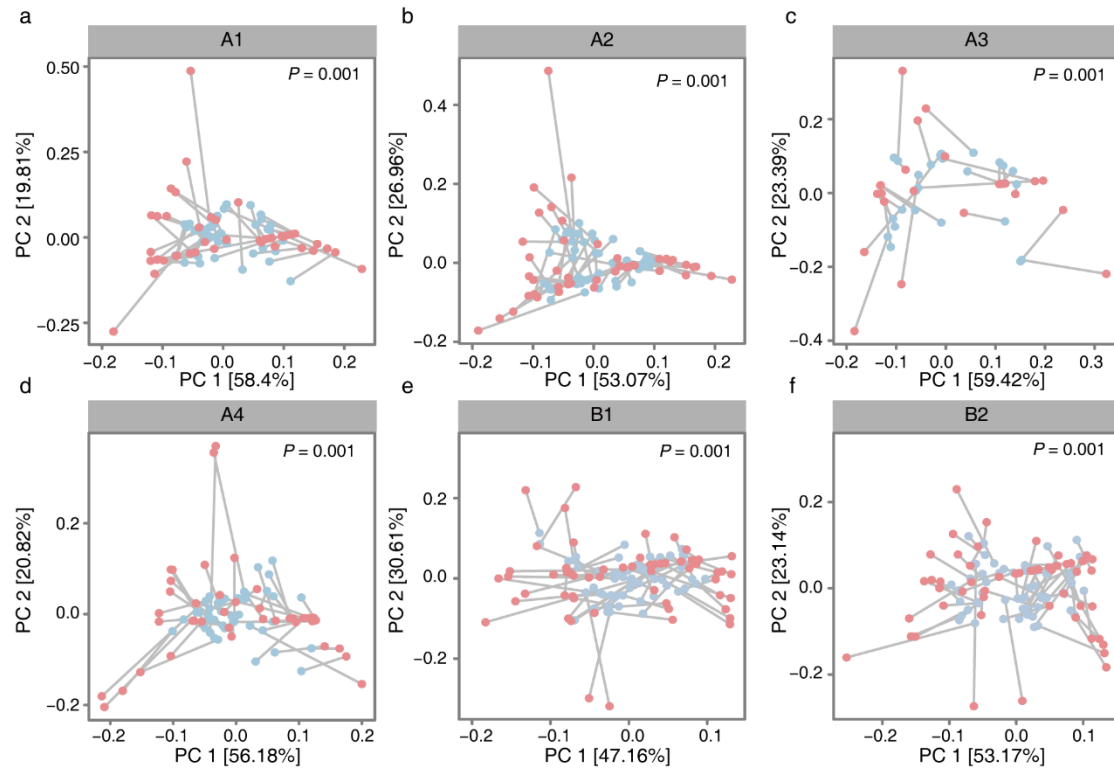
Supplementary Figure 2 | Social group structures the gibbon gut microbiome. The significance of differences in the alpha diversity of (a) Shannon, (b) evenness, (c) observed richness, and (d) phylogenetic diversity between different social groups were identified by the Wilcoxon rank-sum test. (e) Principal coordinate analysis (PCoA) of all gibbon gut microbiome samples based on the Bray-Curtis distance matrix. Analysis of similarity (ANOSIM) statistics considered samples grouped by social groups. Colors denoted different social groups (orange, NK; purple, BC). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.



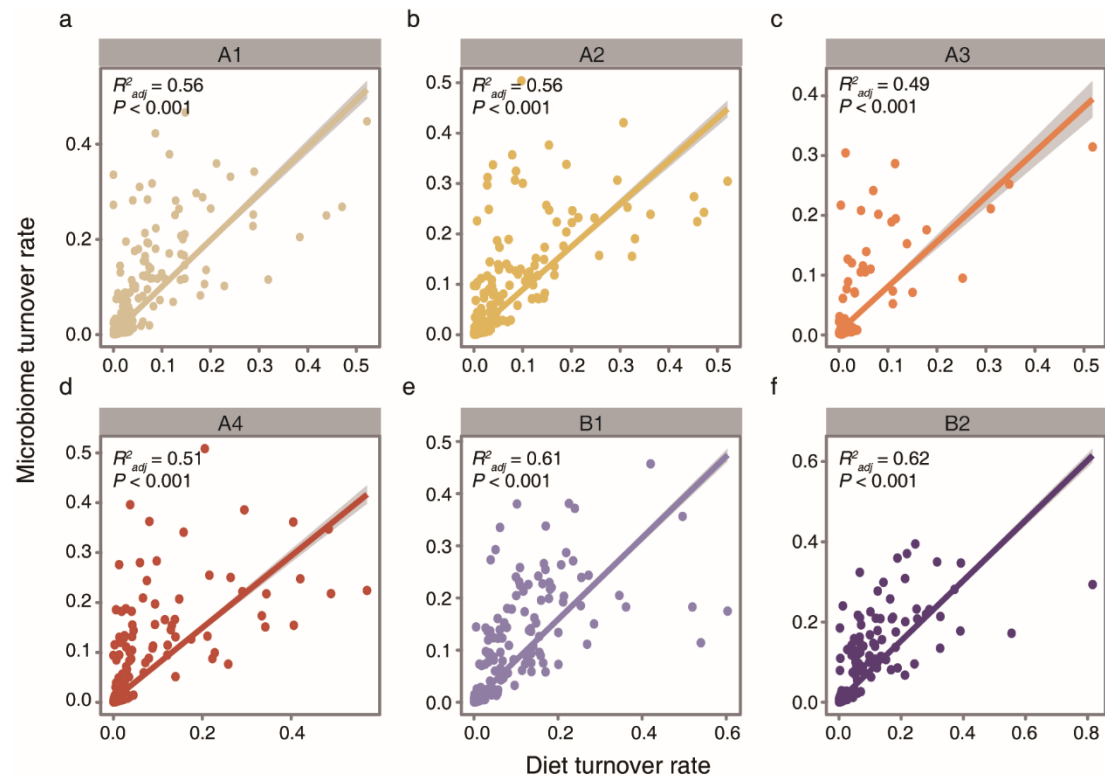
Supplementary Figure 3 | Significant correlations between alpha diversity and diet composition across social groups (orange, NK; purple, BC). The significant correlations between the Shannon Index, Community Evenness, Observed Richness, and Faith Diversity and the proportion of fruits (**a, c, e, g**) and leaf (**b, d, f, h**) were presented. Grey shadow represented the 95% confidence interval. The adjusted R^2 values for linear regression were presented. The p -values were obtained from linear regression modeling.



Supplementary Figure 4 | Relationship between dissimilarities of microbiome structure and the diet was evaluated by Mantel's correlation. Color-coded best-fit lines and p -values were obtained by Mantel's test.

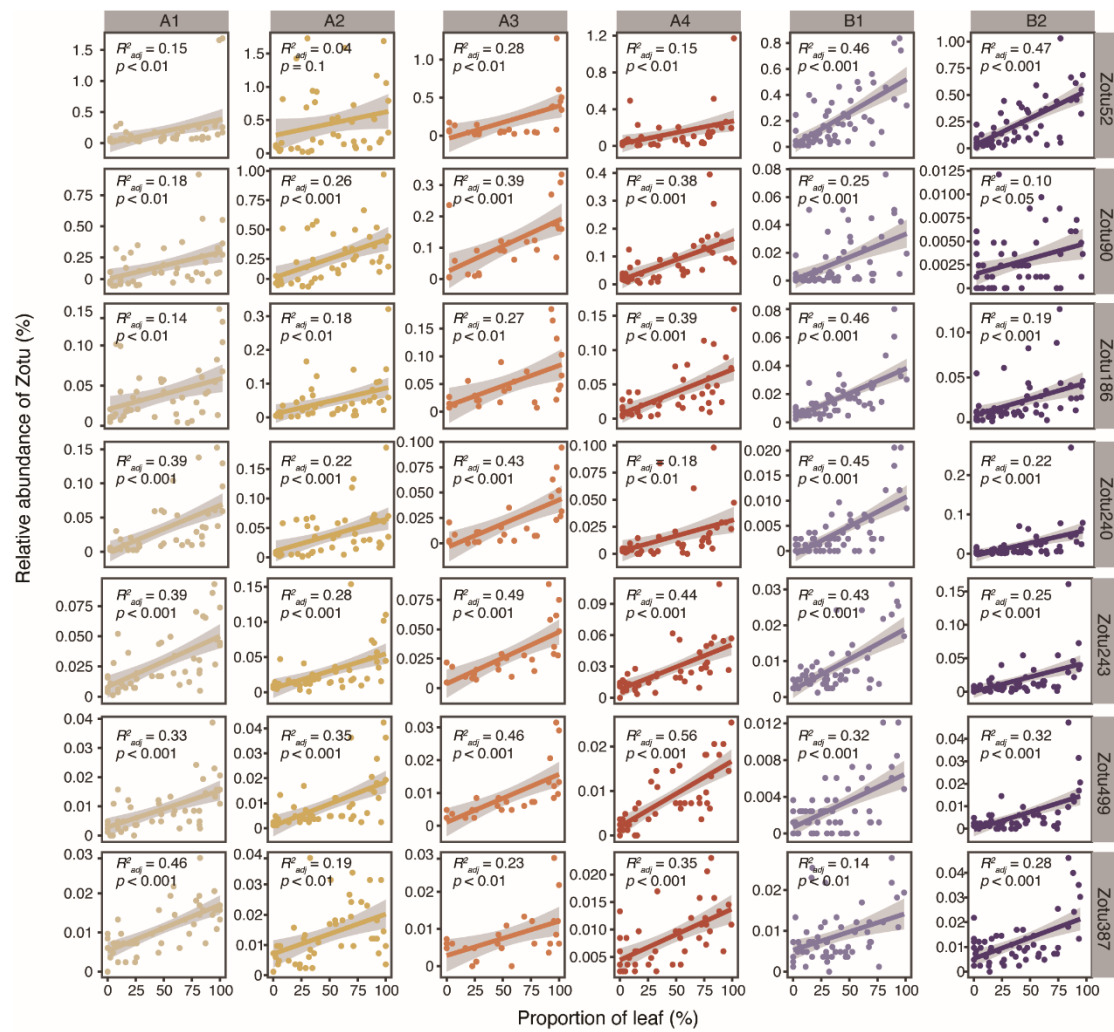


Supplementary Figure 5 | Procrustes analyses to visualize how each individual's seasonal diet and microbiome composition map to each other. (a-f) Procrustes rotated the results of separate PCA of diet composition (red circle) and gut microbiota composition (blue circle). The line showed correspondence between diet and microbiome of each sample. The significant dissimilarity between diet and microbiome ordinations was tested by the Monte Carlo p -value.

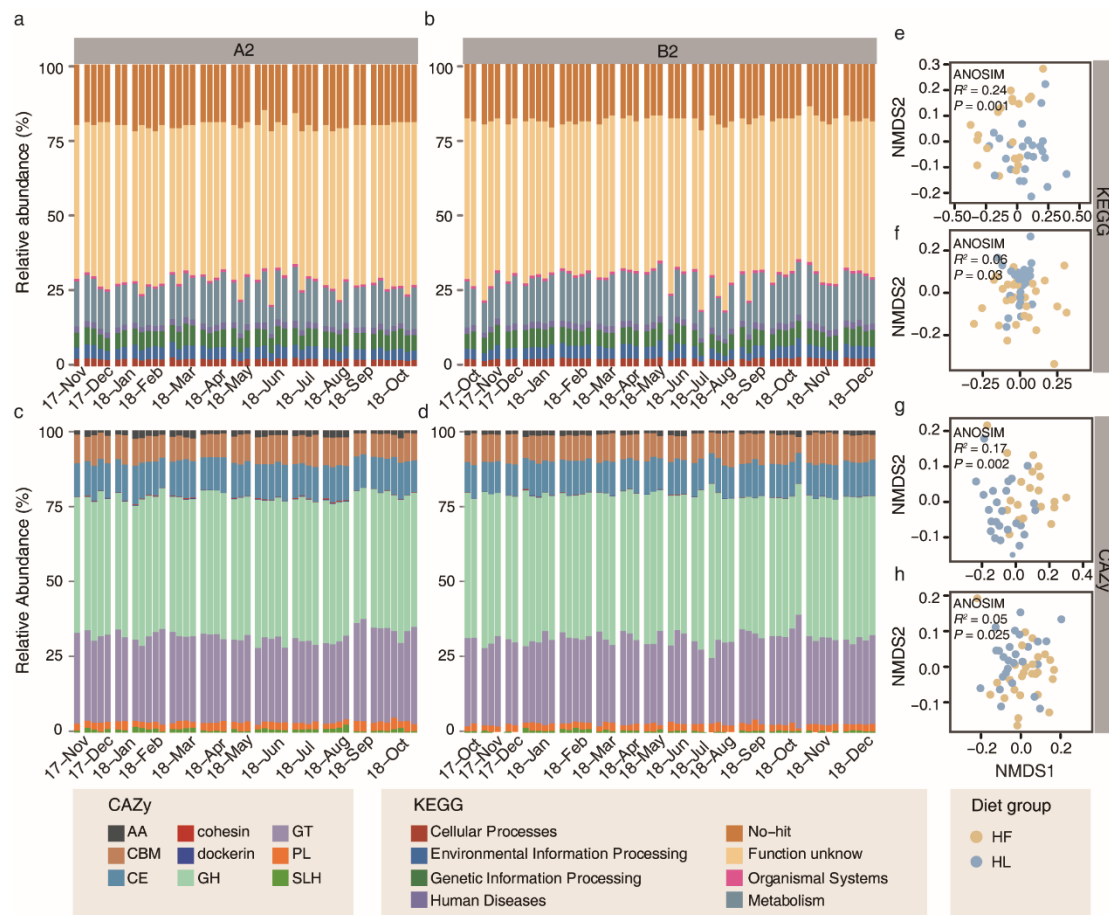


Supplementary Figure 6 | Season turnover of gut microbiota across individuals.

(a-f) The significant correlations between the seasonal turnover rate in diet and microbiome composition across different gibbon individuals were shown. Different colors corresponded to individuals in the top panel. The adjusted R^2 values for linear regression were presented. The p -values were obtained from linear regression modeling.

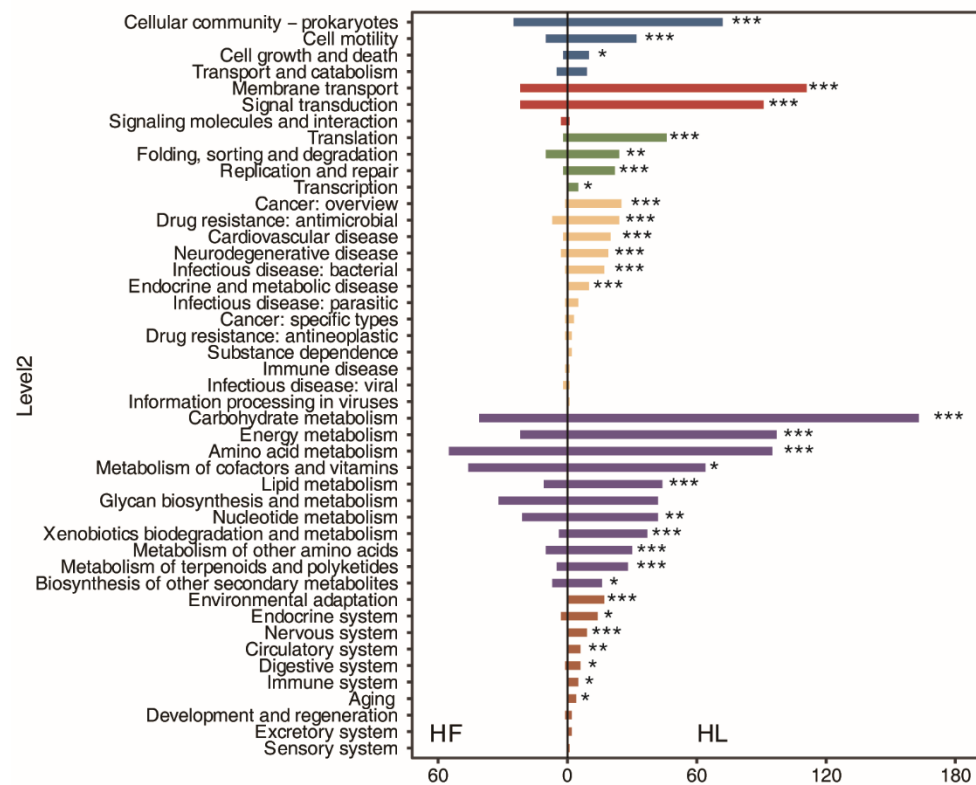


Supplementary Figure 7 | Significant correlations between the relative abundances of seven ZOTUs and the diet with only significant correlations across all individuals were shown. The lines and confidence intervals were obtained from the linear regression. The adjusted R^2 values for linear regression were presented. The p -values were obtained from linear regression modeling.

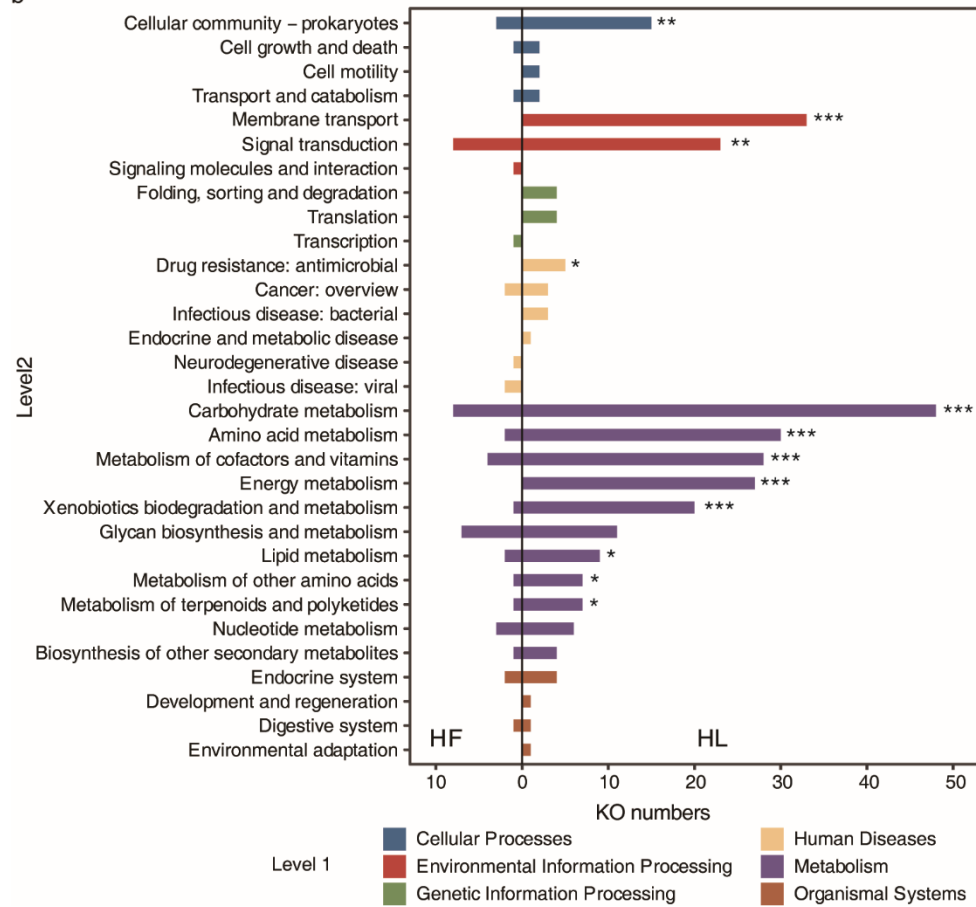


Supplementary Figure 8 | Longitudinal functional profiles of two gibbons. Relative abundance of (a and b) KO categories and (c and d) CAZymes families across two gibbons (a and c, A2; b and d, B2). NMDS plot of Bray-Curtis dissimilarity in two gibbon individuals (e and g, A2; f and h, B2) based on the functional profiles of (e and f) KO categories and (g and h) CAZymes families. Analysis of similarity (ANOSIM) statistics considered samples grouped by different diet types. Colors denoted different diet types (yellow, high-fruit; blue, high-leaf).

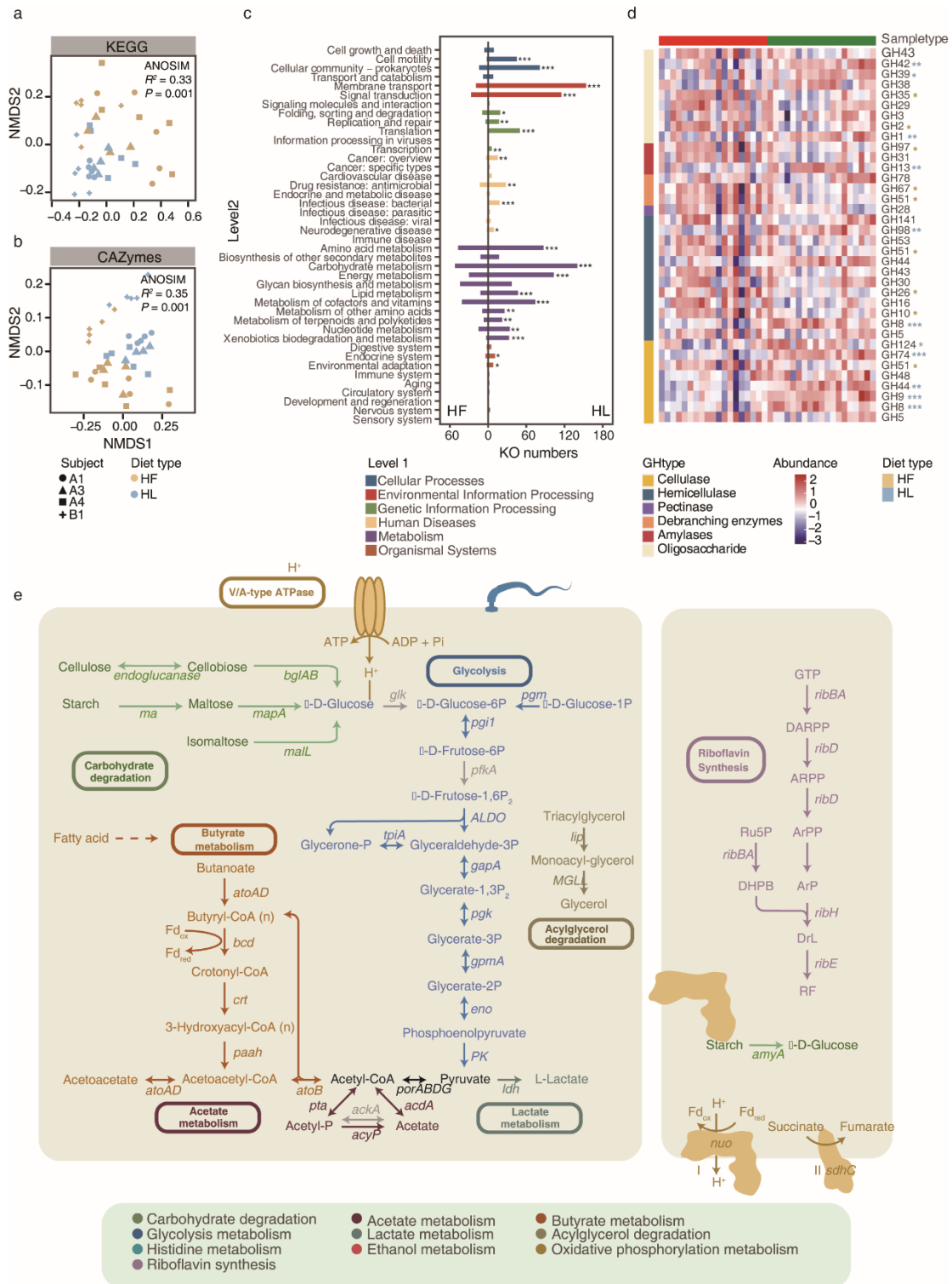
a



b

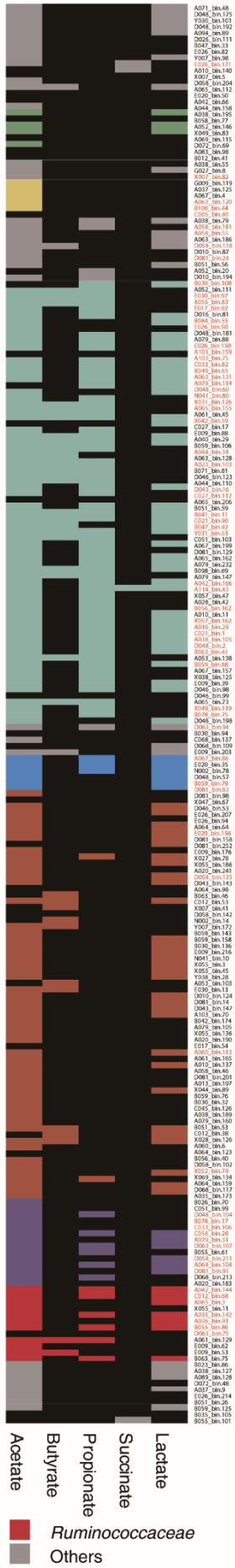


Supplementary Figure 9 | Enrichment of the functional profiles of gibbon microbiomes in different diet types. Significant differences of KEGG Orthology (KO) categories (Level 2) between HF and HF diet types in two gibbon individuals (**a**, A2; **b**, B2). The p -values generated from two-sided Fisher's exact test (10,000 bootstrap replicates) were indicated as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$.



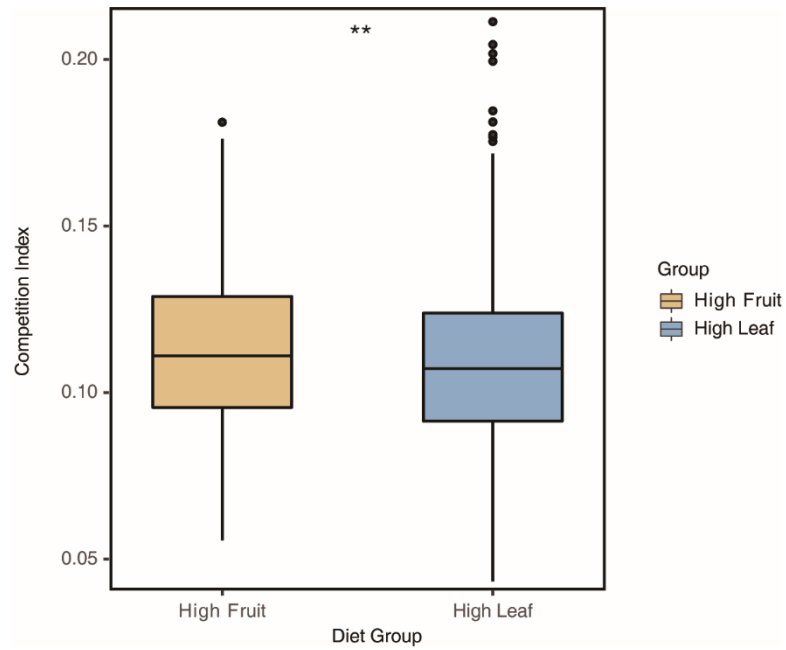
Supplementary Figure 10 | Overview of functional profiles response to different diet types. NMDS plot with Bray-Curtis dissimilarity based on the functional profiles of **(a)** KO categories and **(b)** CAZymes families. Different colors represented samples in distinct dietary types and shapes indicated different subjects. Enrichment of

functional profiles of gibbon microbiomes in different diet types (c). The p values generated from two-sided Fisher's exact test (10,000 bootstrap replicates) were indicated as follows: $*p < 0.05$; $**p < 0.01$; $***p < 0.001$. Heatmap of the CAZymes profile based on the standardized relative abundances of each CAZymes family (d). CAZymes associated with cellulase, hemicellulase, pectinase, debranching enzymes, amylases, and oligosaccharide degradation were presented. CAZymes families with significant enrichment in the leaf- and fruit-dominated periods were marked. The detailed information on the genes was summarized in Supplementary Table 7. Overview of functional profiles depicting the pathways enriched in high-leaf (left) and high-fruit (right) periods (e). The colors of different solid lines corresponded to different metabolic pathways. The gray lines indicated the absence of pathways. The detailed information on the genes was summarized in Supplementary Table 8.

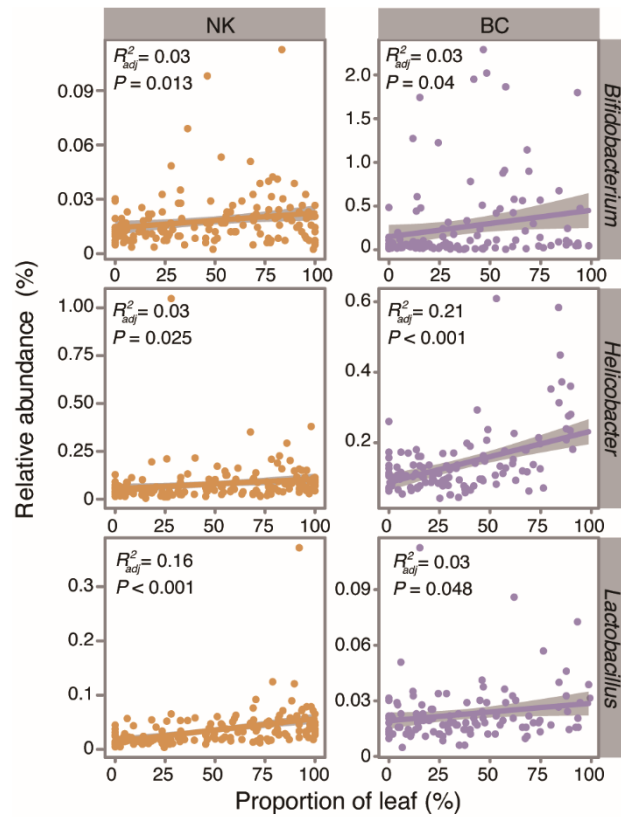


AD01_bln48
D046_bln75
Y030_bln103
D046_bln103
AD04_bln89
D025_bln111
B047_bln33
F036_bln82
Y007_bln98
C008_bln71
AD15_bln140
X007_bln5
D046_bln204
AD05_bln12
F040_bln50
AD02_bln65
D046_bln108
AD08_bln125
F058_bln19
AD02_bln146
X049_bln83
AD05_bln115
D072_bln69
AD02_bln98
B012_bln41
AD02_bln55
D027_bln6
X007_bln62
D049_bln19
AD07_bln25
AD07_bln4
B002_bln40
B100_bln44
C005_bln64
AD08_bln79
AD05_bln181
AD05_bln16
AD05_bln186
D046_bln118
D015_bln47
D081_bln24
B001_bln66
AD02_bln10
D070_bln198
AD02_bln111
X025_bln83
B017_bln62
D016_bln81
F044_bln55
D046_bln108
D046_bln181
AD07_bln88
C008_bln100
A103_bln159
A103_bln72
C018_bln72
X049_bln83
AD05_bln18
AD07_bln114
D046_bln108
B041_bln80
B011_bln146
AD05_bln55
AD01_bln45
C012_bln1
C027_bln17
C027_bln17
F009_bln88
AD05_bln19
B059_bln106
AD05_bln106
AD02_bln103
AD02_bln103
D046_bln123
D044_bln10
D042_bln10
C027_bln12
AD05_bln206
B001_bln59
B041_bln11
C027_bln100
B047_bln43
X021_bln53
X051_bln10
AD07_bln109
AD01_bln79
AD05_bln162
AD09_bln232
B098_bln89
AD09_bln47
AD12_bln166
A114_bln1
X057_bln47
AD05_bln42
F056_bln162
AD10_bln11
AD10_bln162
AD10_bln9
AD10_bln105
D046_bln105
D046_bln105
D043_bln41
AD05_bln138
AD05_bln103
AD07_bln125
X038_bln125
D009_bln39
D046_bln98
D046_bln99
AD05_bln71
F040_bln19
D046_bln198
B030_bln84
D046_bln137
D046_bln109
F009_bln103
F027_bln36
F020_bln35
AD02_bln78
D046_bln17
F009_bln79
D081_bln86
X045_bln86
X047_bln27
X046_bln83
F026_bln207
D046_bln104
AD04_bln64
C005_bln46
D081_bln158
D081_bln222
F009_bln176
F027_bln78
X055_bln86
AD02_bln441
X058_bln10
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D113_bln53
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D046_bln143
X002_bln14
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B059_bln18
B030_bln136
D006_bln16
B041_bln10
X055_bln6
Y030_bln28
X055_bln103
F030_bln13
D010_bln14
D081_bln14
D041_bln147
A102_bln70
D042_bln174
AD05_bln105
X055_bln36
AD02_bln190
B117_bln44
X055_bln1
AD01_bln165
AD10_bln137
AD05_bln46
D081_bln201
AD15_bln197
X044_bln69
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B030_bln12
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X026_bln146
AD05_bln6
AD04_bln123
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D058_bln102
C013_bln4
X009_bln134
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D046_bln117
AD05_bln73
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B078_bln17
C013_bln46
C054_bln18
D046_bln107
D063_bln107
B055_bln81
D056_bln211
AD04_bln104
D081_bln21
D046_bln113
AD02_bln183
X042_bln44
C012_bln68
AD05_bln1
X055_bln1
AD05_bln12
AD05_bln12
B055_bln86
B055_bln36
AD01_bln129
F009_bln62
F009_bln53
B063_bln75
B023_bln86
AD08_bln27
AD09_bln28
AD07_bln46
AD07_bln14
B051_bln14
B059_bln125
B055_bln105
B055_bln101

Supplementary Figure 11 | Metabolic reconstruction of each MAGs enriched in high-leaf diets. Heatmap showed the presence or absence of the abilities for major polysaccharides degradation, sugar utilization, and fermentation. The phylogeny (family level) of the MAGs inferred from GTDB-Tk were indicated with different colors. The MAGs with 12 abilities detected (21 considered in total) were labeled in red. The detailed information was provided in Supplementary Table 5.



Supplementary Figure 12 | The boxplot showed the average competition index among the MAGs enriched in the high-leaf diet during different periods (yellow: high fruit; blue: high leaf). The metabolic interaction indices were inferred from CarMe and RevEcoR. The p -value was obtained from the Wilcoxon rank-sum test. $*p < 0.05$; $p < 0.01$; $***p < 0.001$.**



Supplementary Figure 13 | Correlations between the health-related bacterial taxa (family-level) and the diet. The lines and confidence intervals were obtained from the linear regression. Grey shadow represented the 95% confidence interval. Colors denoted different social groups (orange, NK; purple, BC). The adjusted R^2 values for linear regression were presented. The p -values were obtained from linear regression modeling.

Supplementary Table 1 The abundance of the core ZOTUs appears in over 90% of the samples.

Supplementary Table 2 SIMPER analysis results display the top 10 ZOTUs responsible for the dissimilarity between two social groups.

Supplementary Table 3 Information of diet-responsive ZOTUs.

Supplementary Table 4 The enrichment result of MAGs in different diet

Supplementary Table 5 The summarized taxonomic and metabolic potentials of the enriched MAGs in high-leaf periods.

Supplementary Table 6 Validation of predictive models for relative abundances of dominant microbial taxa.

Supplementary Table 7 Metadata of the gut microbiome samples of gibbons.

Supplementary Table 8 Taxonomic classification of ZOTUs.

Supplementary Table 9 The abundance of selected KEGG pathways in Fig 5 and Supplementary Figure 10.

Supplementary Table 10 The abundance of GH families associated with carbohydrate degradation.