Supplementary Information

Barley extrudates modulate the gut microbiome–metabolome axis *in vitro* through β-glucan fermentation and polyphenol biotransformation.

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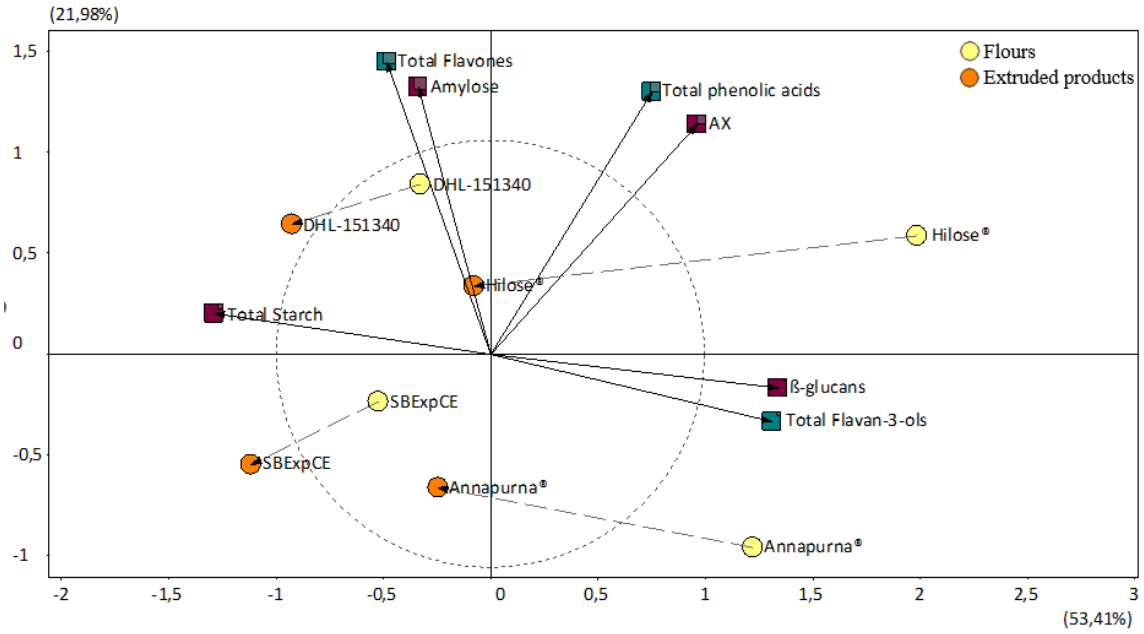
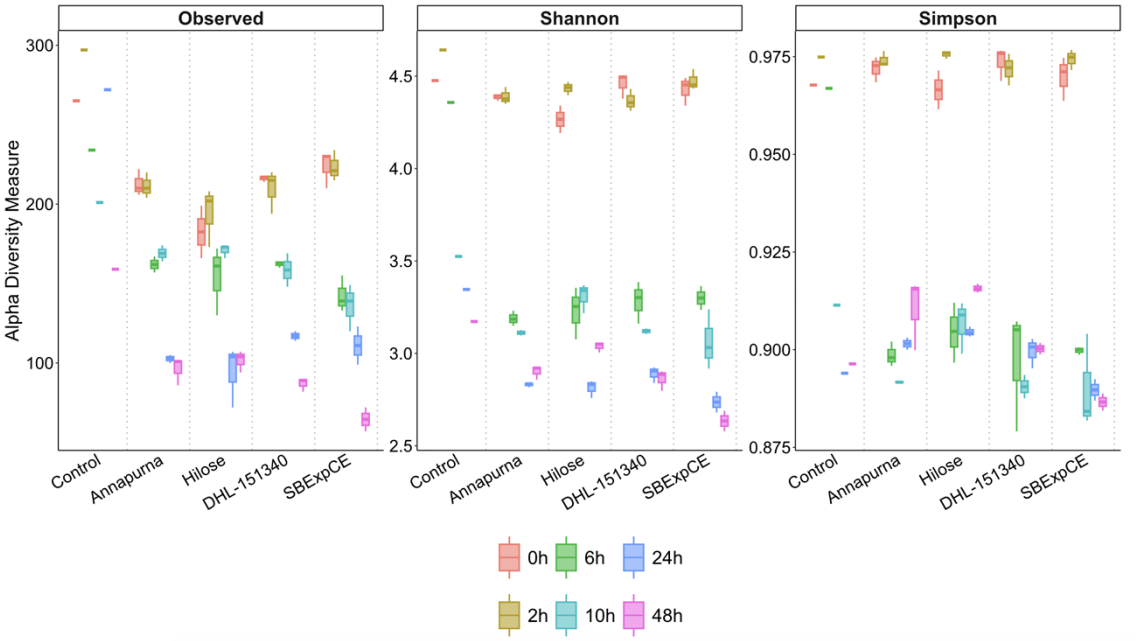
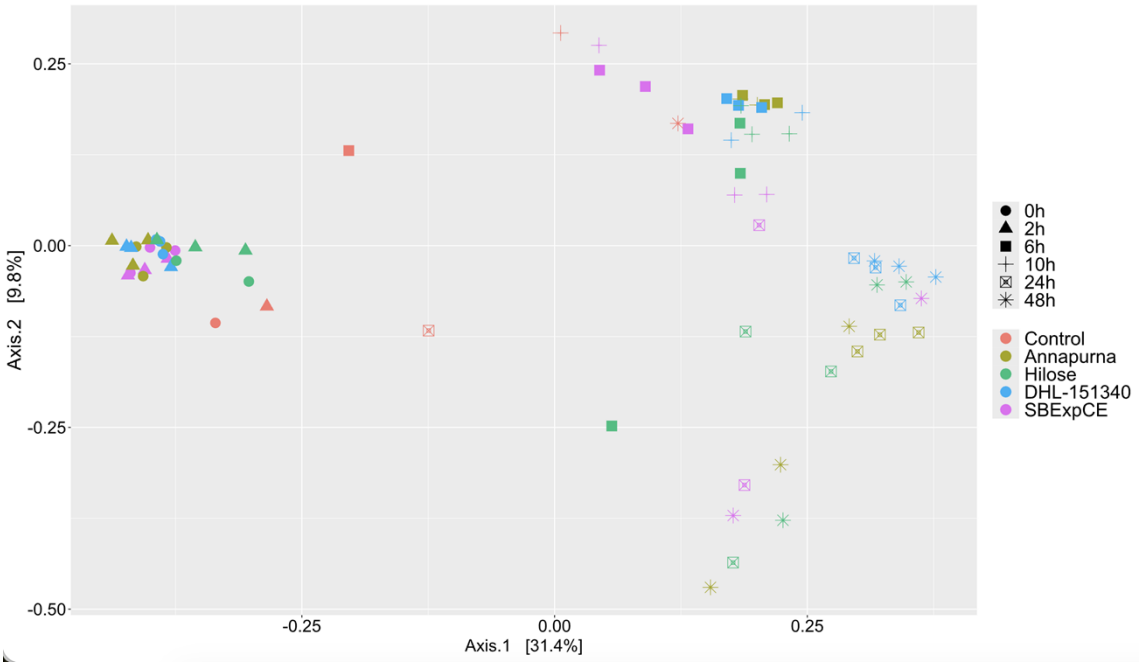


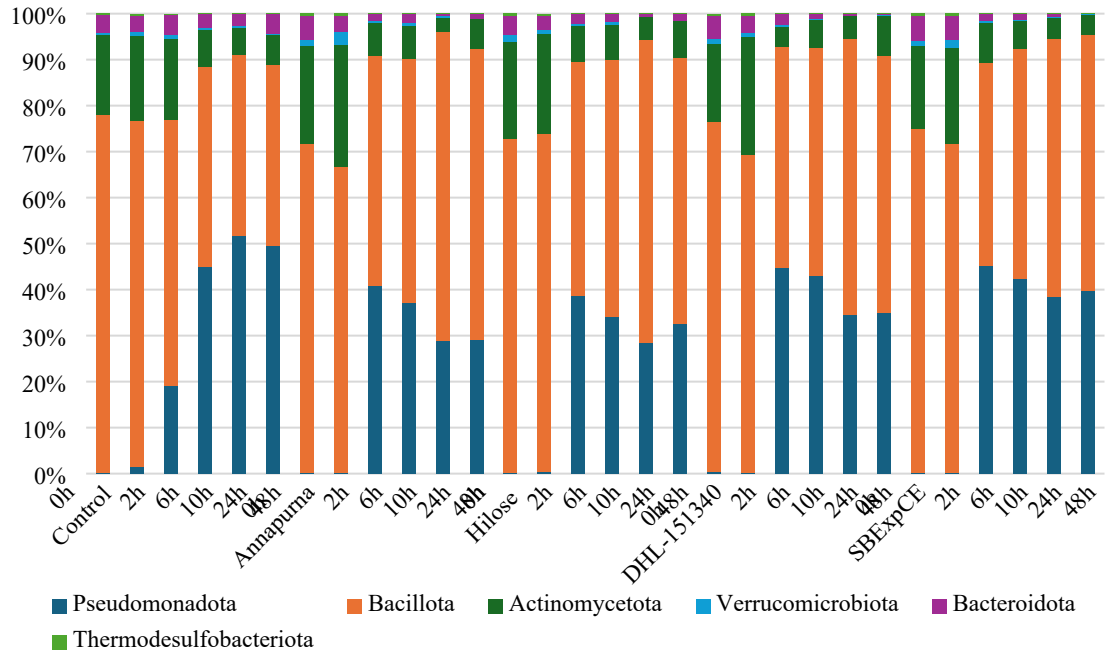
Supplementary Figure 1. Principal component analysis (PCA) of chemical composition variables in barley flours (light circles) and extruded products (dark circles) from four genotypes. Carbohydrate-related variables (β-glucans, arabinoxylans (AX), amylose, and total starch) are represented by purple squares, whereas phenolic compounds (flavan-3-ols, phenolic acids, and flavones) are shown as turquoise squares. PC1 and PC2 explain 53.4% and 22.0% of the total variance, respectively.



Supplemental Figure 2A. Alpha Diversity microbiota analyses of fecal samples. Least mean squares of alpha-diversity indices (Observed; Shannon and Simpson) of fecal samples presented by time (0h, 2h, 6h, 10h, 24h, 48h) and by diet. Annapurna, Hilose, DHL-151340 and SBExpCE vs control (with water and enzymes). Error bars represent standard errors.

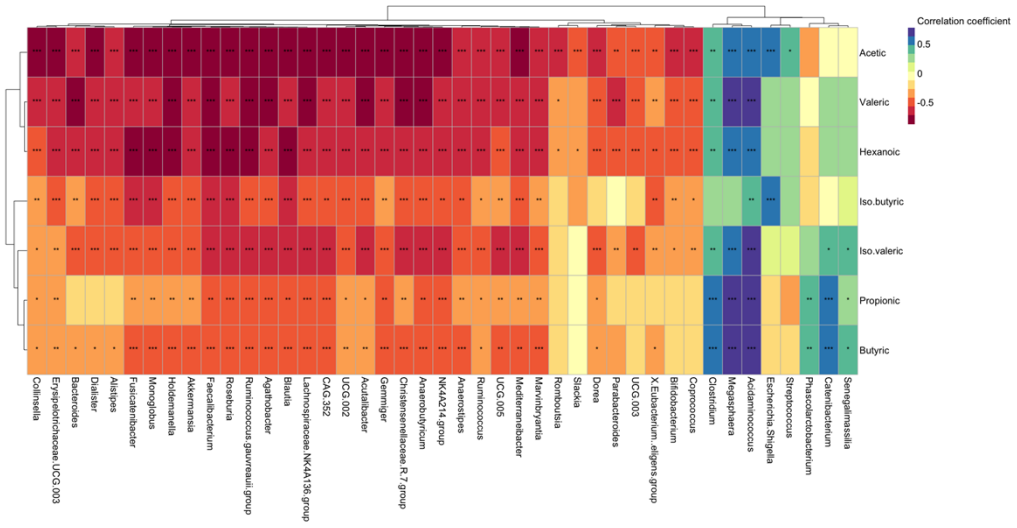


Supplemental Figure 2B. Beta Diversity microbiota analyses of fecal samples. Least mean squares of alpha-diversity indices (Observed; Shannon and Simpson) of fecal samples presented by time (0h, 2h, 6h, 10h, 24h, 48h) and by diet. Annapurna, Hilose, DHL-151340 and SBExpCE vs control.

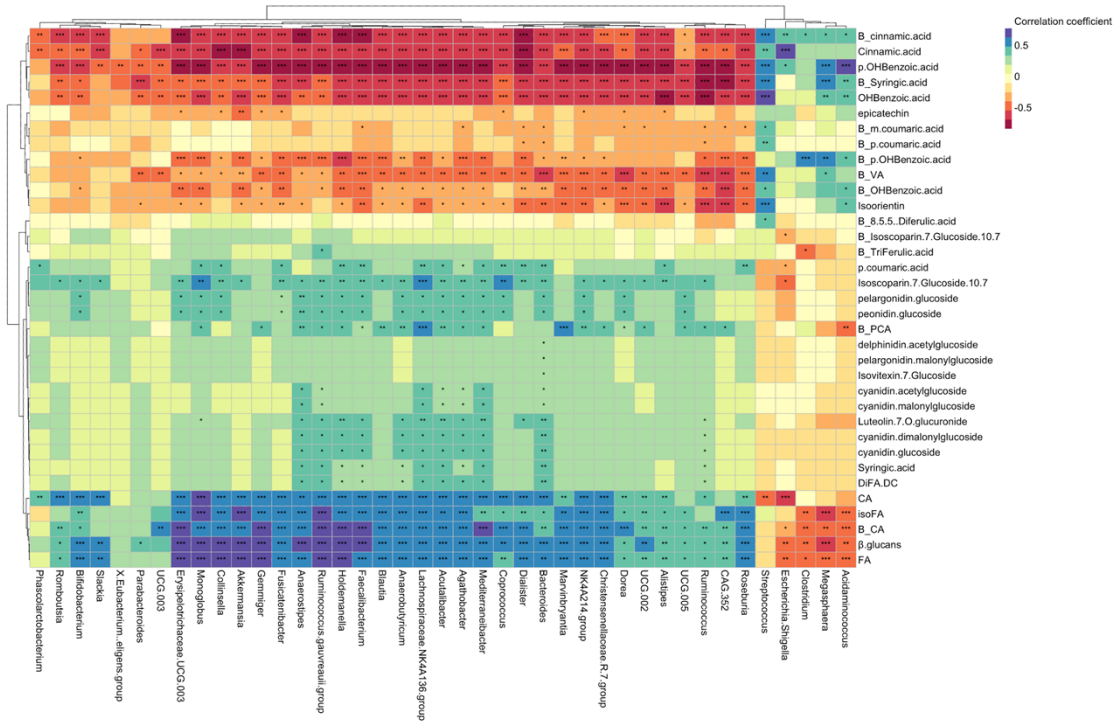


Supplementary Figure 3. Evolution at phylum level of main microbial groups depending on barley and time of fermentation. Data is expressed as mean of relative abundance at 0, 2, 6, 10, 24 and 48h.

A)


Correlation coefficient
0.5
0
-0.5
Acetic
Valeric
Hexanoic
Iso.butyric
Iso.valeric
Propionic
Butyric
Collinsella
Erysipelotrichaceae UCG.003
Bacteroides
Dialister
Alistipes
Fusicatenibacter
Monoglobus
Holdemanella
Akkermansia
Faecalibacterium
Roseburia
Ruminococcus gauvreauii group
Agathobacter
Blautia
Lachnospiraceae NK4A136 group
CAG.352
UCG.002
Acutalibacter
Gemmiger
Christensenellaceae R.7 group
Anaerobutyricum
NK4A214 group
Anaerostipes
Ruminococcus
UCG.005
Mediterraneibacter
Marvinbryantia
Romboutsia
Slackia
Dorea
Parabacteroides
UCG.003
X.Eubacterium.eligens.group
Bifidobacterium
Coprococcus
Clostridium
Megasphaera
Acidaminococcus
Escherichia.Shigella
Streptococcus
Phascolarctobacterium
Catenibacterium
Senegalimassilia

B)


Correlation coefficient
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0
-0.5
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Cinnamic.acid
p.OHBenzoic.acid
B_Syringic.acid
OHBenzoic.acid
epicatechin
B_m.coumaric.acid
B_p.coumaric.acid
B_p.OHBenzoic.acid
B_VA
B_OHBenzoic.acid
Isoorientin
B_8.5.5..Diferulic.acid
B_Isoscoparin.7.Glucoside.10.7
B_TriFerulic.acid
p.coumaric.acid
Isoscoparin.7.Glucoside.10.7
pelargonidin.glucoside
peonidin.glucoside
B_PCA
delphinidin.acetylglucoside
pelargonidin.malonylglucoside
Isovitexin.7.Glucoside
cyanidin.acetylglucoside
cyanidin.malonylglucoside
Luteolin.7.O.glucuronide
cyanidin.dimalonylglucoside
cyanidin.glucoside
Syringic.acid
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CA
isoFA
B_CA
β.glucans
FA
Phascolarctobacterium
Romboutsia
Bifidobacterium
Slackia
X.Eubacterium..eligens.group
Parabacteroides
UCG.003
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Monoglobus
Collinsella
Akkermansia
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Fusicatenibacter
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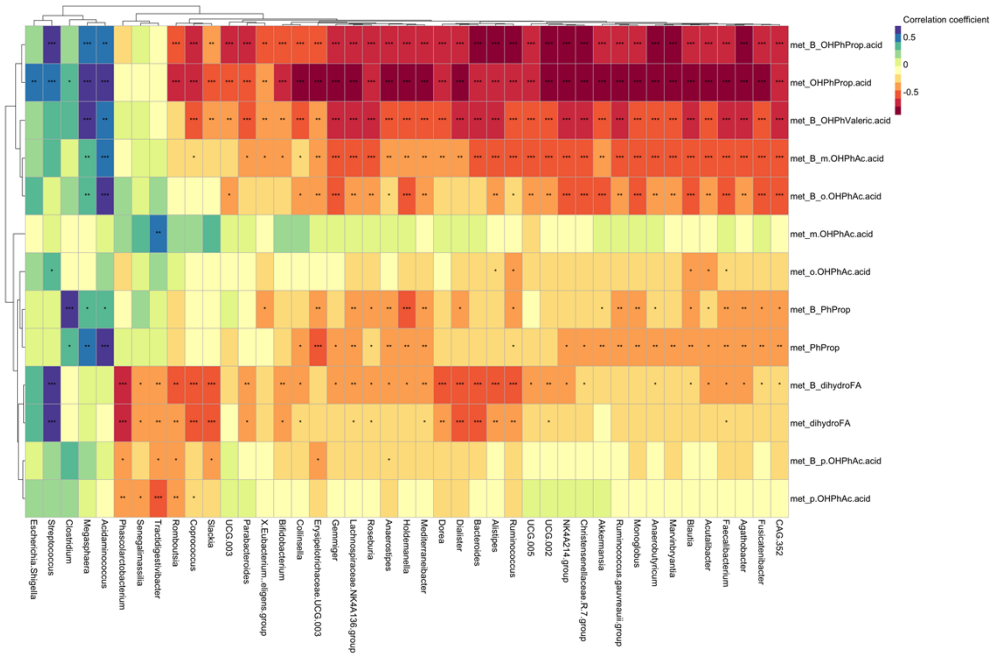
Acutalibacter
Agathobacter
Mediterraneibacter
Coprococcus
Dialister
Bacteroides
Marvinbryantia
NK4A214.group
Christensenellaceae.R.7.group
Dorea
UCG.002
Alistipes
UCG.005
Ruminococcus
CAG.352
Roseburia
Streptococcus
Escherichia.Shigella
Clostridium
Megasphaera
Acidaminococcus

C)



Supplementary Figure 4: A) Associations between phenolic compounds and colonic microbial taxa; B) Associations between SCFAs and colonic microbial taxa; C) Associations between phenolic metabolite profiles and colonic microbial taxa. Compounds with the prefix B- correspond to phenolic compounds from the bound fraction. **IsoFA**: 3'-Hydroxy-4'-methoxycinnamic acid (cis/trans); **FA**: 4'-Hydroxy-3'-methoxycinnamic acid (cis/trans); **cyanidin.acetylglucoside**: Cyanidin-*O*-Acetylglucoside; **cyanidin.malonylglucoside**: Cyanidin-*O*-Malonylglucoside; **cyanidin.dimalonylglucoside**: Cyanidin-*O*-Dimalonylglucoside; **cyanidin.glucoside**: Cyanidin-3-*O*-Glucoside; **B_PCA**: B_3,4-Dihydroxybenzoic acid; **peonidin.glucoside**: Peonidin-*O*-Glucoside; **B_m-Coumaric acid**: B_3'-Hydroxycinnamic acid (cis/trans); **B_p-Coumaric acid**: B_4'-Hydroxycinnamic acid (cis/trans); **B_p-OHBenzoic acid**: B_4-Hydroxybenzoic acid; **B_OHBenzoic acid**: B_Hydroxybenzoic acid; **B_VA**: B_4-Hydroxy-3-methoxybenzoic acid; **p-OHBenzoic acid**: 4-Hydroxybenzoic acid; **B_Syringic acid**: B_3,5-Dimethoxy-4-hydroxybenzoic acid; **OHBenzoic acid**: Hydroxybenzoic acid; **OHPhProp acid**: Hydroxyphenylpropanoic acid; **DiHydroFA:** 3-(4'-Hydroxy-3'-methoxyphenyl)propanoic acid; **p.OHPhAc acid**: 4'-Hydroxyphenylacetic acid; **m.OHPhAc acid:** 3'-Hydroxyphenylacetic acid; **PhProp**: Phenylpropanoic acid; **o.OHPhAc acid**: 2'-Hydroxyphenylacetic acid; **OHPhValeric acid**: Hydroxyphenylvaleric acid.