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Article

The Gut Microbiota-Immunity Axis in ALS: A Role in Deciphering Disease Heterogeneity?

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Table S1. Low Limit of Quantification (LLOQ, pg/ml) for each evaluated cytokine.

Cytokine	LLOQ (pg/ml)
G-CSF	13.00
IFN-γ	7.47
IL-1β	2.05
IL-2	4.27
IL-4	11.00
IL-6	8.15
IL-8	2.17
IL-10	2.25
IL-15	3.05
IL-17A	1.81
MCP-1	4.76
MIP-1α	1.56
TNFα	6.52
VEGF-A	1.19
IL-27	24.00
IL-5	6.42
IP-10	2.15
IL-12p70	6.40
IL-13	2.15
GM-CSF	13.00
IFN-α	0.55
IL-9	7.69
P-selectin	1233.00
IL-1α	0.54
IL-23	14.00

IL-18	6.84
IL-21	7.79
sICAM1	212.00
IL-22	26
E-selectin	250.00

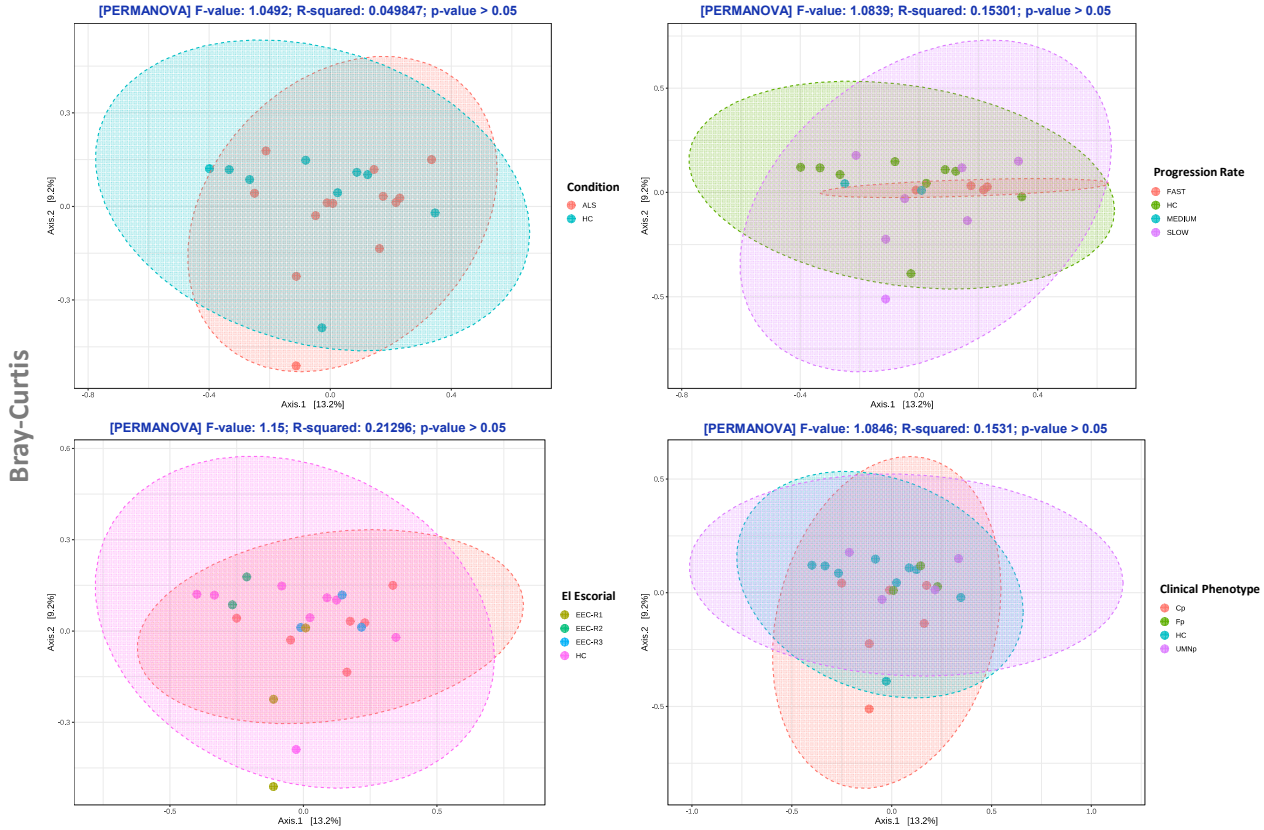

Figure S1. Principal coordinates analysis (PCoA) according to the Bray-Curtis beta-diversity metric. Results of the permutational multivariate analysis of variance (PERMANOVA) are also shown. Subjects are colored according to the group: a) condition (ALS patients vs healthy controls), b) rate of ALS progression c) El Escorial Criteria d) clinical phenotype. ALS= amyotrophic lateral sclerosis. EEC= EL Escorial Criteria; EEC-R0= definite; EEC-R1=clinically probable; EEC-R2=probable-laboratory supported, EEC-R3= possible. Bp= bulbar phenotype; Cp= Classic phenotype; Fp= Flail arm/leg phenotype; UMNp= Upper Motor Neuron predominant phenotypes.

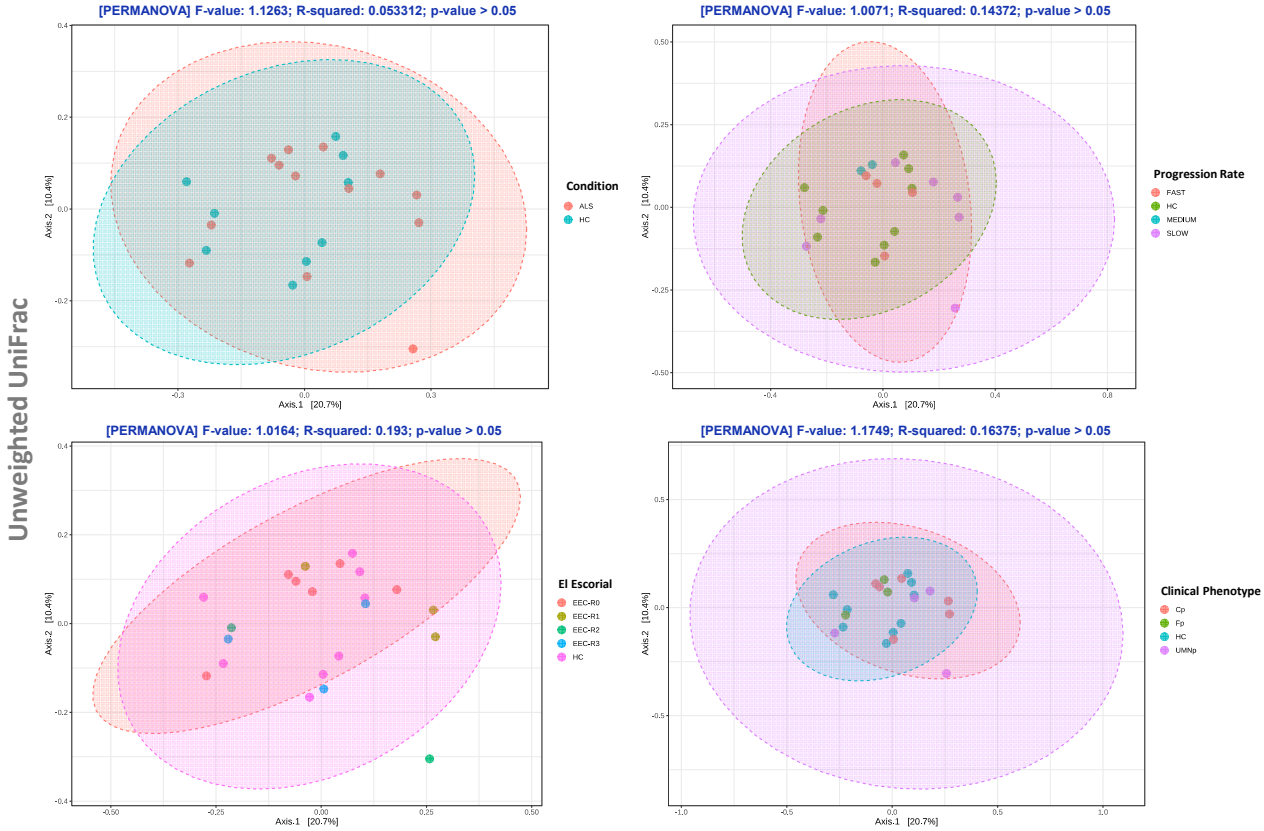

Figure S2. Principal coordinates analysis (PCoA) according to the Unweighted UniFrac beta-diversity metric. Results of the permutational multivariate analysis of variance (PERMANOVA) are also shown. Subjects are colored according to the group: a) condition (ALS patients vs healthy controls), b) rate of ALS progression c) El Escorial Criteria d) clinical phenotype. ALS= amyotrophic lateral sclerosis. EEC= EL Escorial Criteria; EEC-R0= definite; EEC-R1= possible, EEC-R2=clinically probable; EEC-R3=probable-laboratory supported. Cp= Classic phenotype; Fp= Flail arm/leg phenotype; UMNp= Upper Motor Neuron predominant phenotypes.

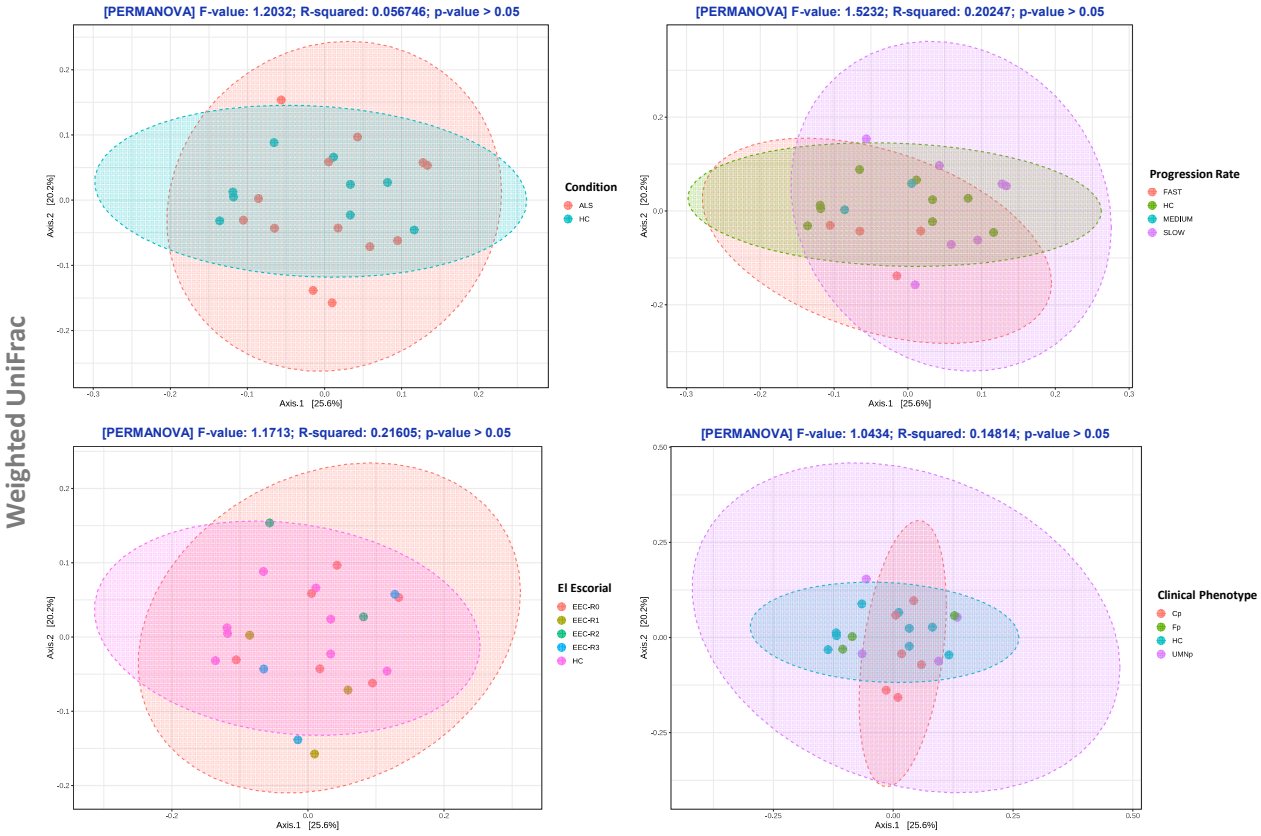

Figure S3. Principal coordinates analysis (PCoA) according to the Weighted UniFrac beta-diversity metric. Results of the permutational multivariate analysis of variance (PERMANOVA) are also shown. Subjects are colored according to the group: a) condition (ALS patients vs healthy controls), b) rate of ALS progression c) El Escorial Criteria d) clinical phenotype. ALS= amyotrophic lateral sclerosis. EEC= EL Escorial Criteria; EEC-R0= definite; EEC-R1= possible, EEC-R2=clinically probable; EEC-R3=probable-laboratory supported. Bp= bulbar phenotype; Cp= Classic phenotype; Fp= Flail arm/leg phenotype; UMNp= Upper Motor Neuron predominant phenotypes.

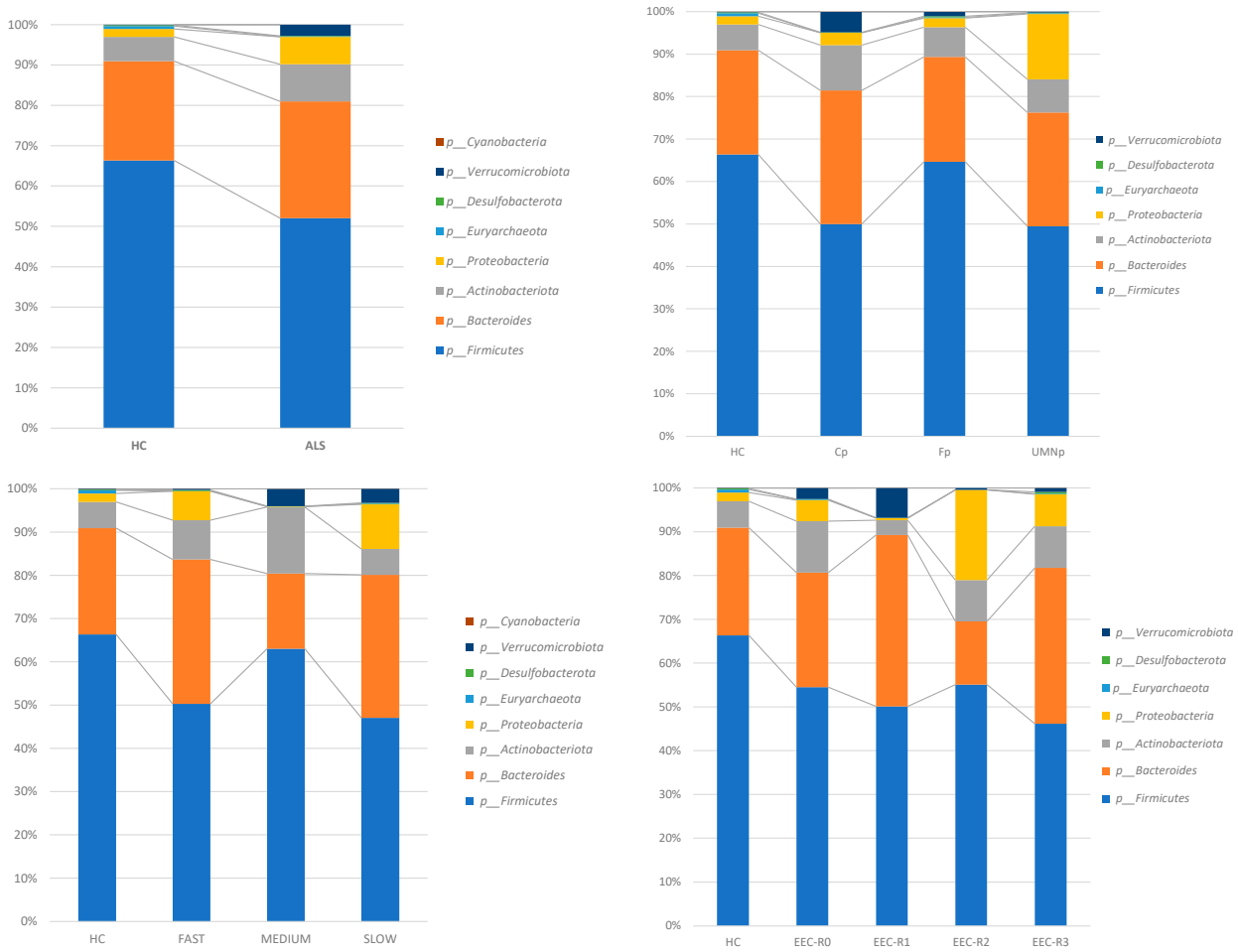

Figure S4. Stacked bar plots displaying the average relative abundance of bacterial amplicon sequence variants (ASVs) identified at the phylum taxonomic level in the gut microbiota of ALS patients' subgroups and healthy controls. Abbreviations are as explained in Figure S1.

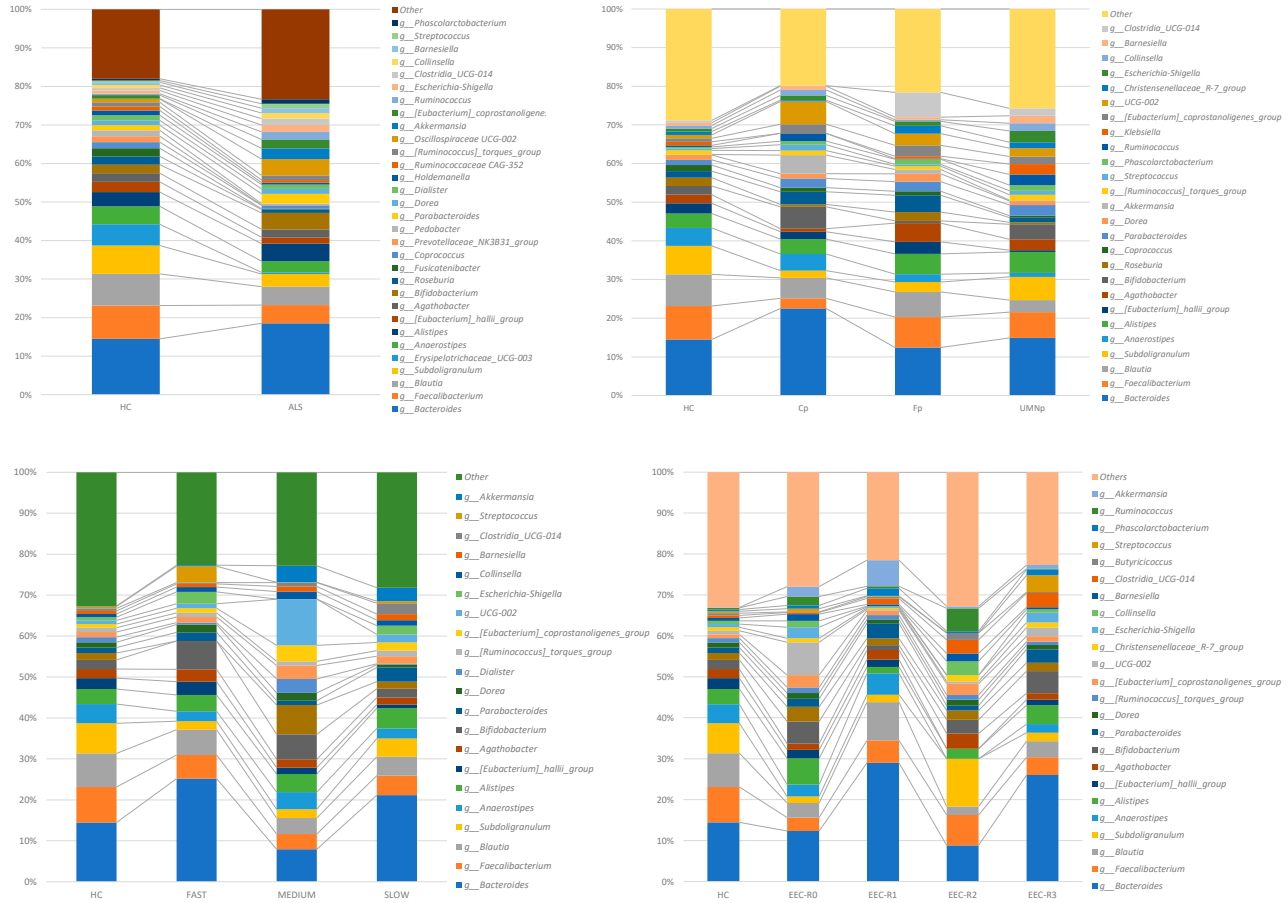

Figure 5S. Stacked bar plots displaying the average relative abundance of bacterial amplicon sequence variants (ASVs) identified at the genus taxonomic level in the gut microbiota of ALS patients' subgroups and healthy controls. Abbreviations are as explained in Figure S1.