Appendix

Alterations of the gut ecological and functional microenvironment in different stages of multiple sclerosis

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Supplementary Figure and Table Legends

Figure S1. Comparison of the microbial abundance between the five subject groups at the phylum and genus levels.

(A) Comparison of the abundance at the phylum level between the five groups based on 16S data. Phyla with an average relative abundance ≥0.1% in at least one group are represented. (B) Comparison of the abundance at the genus level between the five groups based on 16S data. Genera with an average relative abundance ≥0.5% in at least one subject group are represented. Each box plot represents median, interquartile range, minimum, and maximum values. **p* <0.05 based on the Wilcoxon rank sum test with the Benjamini-Hochberg method for multiple group comparisons.

Figure S2. Comparison of the microbial abundance in significantly increased or decreased species/OTUs between the five subject groups.

Comparison of the abundance at the species/OTU level between the five groups based on 16S data. Species/OTUs that were significantly increased or decreased between the five groups are represented. Each box plot represents median, interquartile range, minimum, and maximum values. **p* <0.05, ***p* <0.01, ****p* <0.001, and *****p* <0.0001 based on the Wilcoxon rank sum test with the Benjamini-Hochberg method for multiple group comparisons.

Figure S3. Differences in functional profile of gut microbiome between RRMS and healthy controls.

(A) The list of 97 significantly enriched and 117 significantly depleted KEGG orthologies (KOs) in the RRMS group compared to those in the HC group (Wilcoxon test, *p* <0.05). The Z-score based on the abundance of each KO is depicted from lowest (blue) to highest (red) according to the scale shown at the top. (B) List of KEGG pathways including at least one of the 97 significantly enriched KOs (RRMS-enriched KOs) or one of the 117 significantly depleted KOs (RRMS-depleted KOs) in the comparisons between the HC and RRMS groups. These individual pathways are ranked based on the ratios of RRMS-enriched KOs to -depleted KOs. *p*-value is based on the Fisher's exact test.

Table S1. Demographics and characteristics of the five groups.

Sixty-two patients with RRMS, 15 patients with SPMS, 21 patients with atypical MS, 20 patients with NMOSD, and 55 healthy controls were recruited. Samples from twenty of the 62 RRMS patients were previously subjected to 16S analysis (1). Data are represented as mean ± SEM. **Abbreviations:** BMI = body mass index; ARR = annual relapse rate; EDSS = expanded disability status scale; PSL = prednisolone; IS = immunosuppressive drugs; DMDs; disease modifying drugs; IFN-β = interferon-β; GA = glatiramer acetate; FTY = fingolimod; NTZ = natalizumab; DMF = dimethyl fumarate; HC = healthy control; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis; atypical MS = atypical multiple sclerosis; NMOSD = neuromyelitis optica spectrum disorder. A *p*-value based on comparisons between the RRMS, SPMS, atypical MS, and NMOSD groups (Chi-squared test). B *p*-value based on comparisons between the RRMS, atypical MS, and NMOSD groups (Kruskal-Wallis test).

Table S2. Validation of the differences in gender and immunotherapy based on gut microbiome beta diversity.

Beta diversity indexes based on UniFrac analysis. R^2 and statistical test in permutational multivariate analysis of variance between HCs (female) and HCs (male) and between RRMS patients receiving immunotherapy and RRMS patients not receiving immunotherapy. UniFrac distance was based on the 16S data.

Table S3. Summary of abundance of microbial species at the genus-level.

An average abundance was calculated from 3,000 16S reads. Data are represented as mean ± SEM.

Table S4. The 16S-based significant changes in abundance for all species/OTUs between any two groups among the five subject groups.

Thirty species/OTUs exhibited a significant increase or decrease (Wilcoxon test, $p < 0.05$) between any pair of the five subject groups. The statistical procedure was based on Wilcoxon rank sum test with the Benjamini-Hochberg method for multiple group comparisons. 1 Hyphen indicates ≥97% identity with 16S rRNA gene V1-2 of the corresponding closest known species. 2 G1 / G2 indicates an average relative abundance in the Group 1 (G1) / an average relative abundance in the Group 2 (G2). Read number = 1 was assumed for "not detected" to calculate G1 / G2.

Table S5. Similarity of 16S rRNA gene V1-V2 region for species/OTUs exhibiting significant changes in abundance (RRMS vs HC) between the present paper and our previous paper.

Seventeen species/OTUs (12 mapped clusters and five unmapped OTUs) with significant changes in abundance between RRMS and HC groups in the present paper are shown in blue. Twenty-one species/OTUs (15 mapped clusters and six unmapped OTUs) with significant changes in abundance between the RRMS and HC groups in our previous paper (1) are shown in red. "Mapped CLs" and "rclusts" correspond mapped clusters. "Unmapped_OTUs" and "unmap_OTUs" correspond unmapped OTUs.

Table S6. KEGG orthologies having significant changes in abundance between two of the five groups based on the metagenomic data.

Abbreviations: KO = KEGG orthology; G1 = Group 1; G2 = Group 2.

Table S7. Demographics and characteristics of the three groups in metabolite analysis.

Demographics and characteristics of the HC, RRMS, and SPMS groups with respect to the measurements of fecal metabolites. Data are represented as mean ± SEM. **Abbreviations:** BMI = body mass index; ARR = annual relapse rate; EDSS = expanded disability status scale; HC = healthy control; RRMS = relapsing remitting multiple sclerosis; SPMS = secondary progressive multiple sclerosis.

References

1. S. Miyake *et al.*, Dysbiosis in the Gut Microbiota of Patients with Multiple Sclerosis, with a Striking Depletion of Species Belonging to Clostridia XIVa and IV Clusters. *PLoS One* **10**, e0137429 (2015).

Figure S1

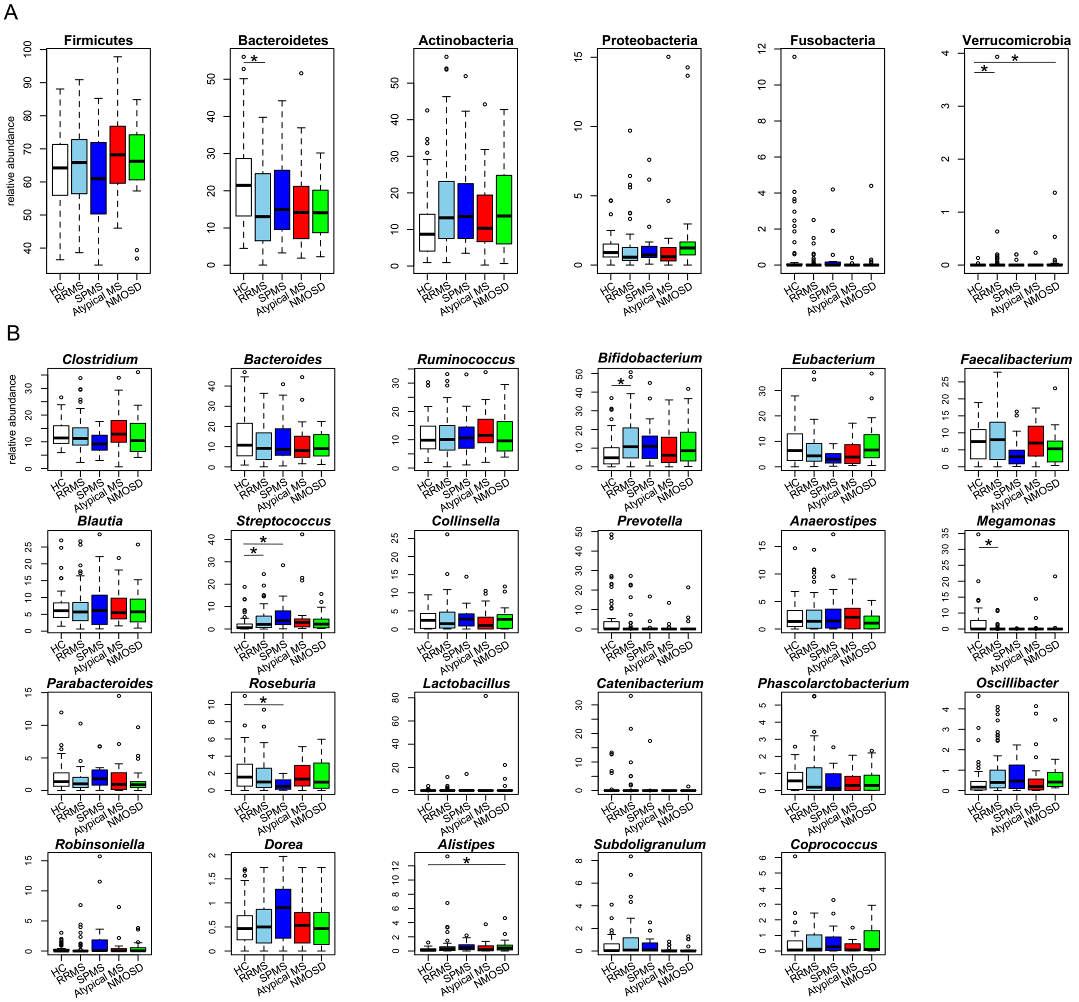

A
Firmicutes
Bacteroidetes
Actinobacteria
Proteobacteria
Fusobacteria
Verrucomicrobia
relative abundance
HC
RRMS
SPMS
Atypical MS
NMOSD
*
B
Clostridium
Bacteroides
Ruminococcus
Bifidobacterium
Eubacterium
Faecalibacterium
Blautia
Streptococcus
Collinsella
Prevotella
Anaerostipes
Megamonas
Parabacteroides
Roseburia
Lactobacillus
Catenibacterium
Phascolarctobacterium
Oscillibacter
Robinsoniella
Dorea
Alistipes
Subdoligranulum
Coprococcus

Figure S2

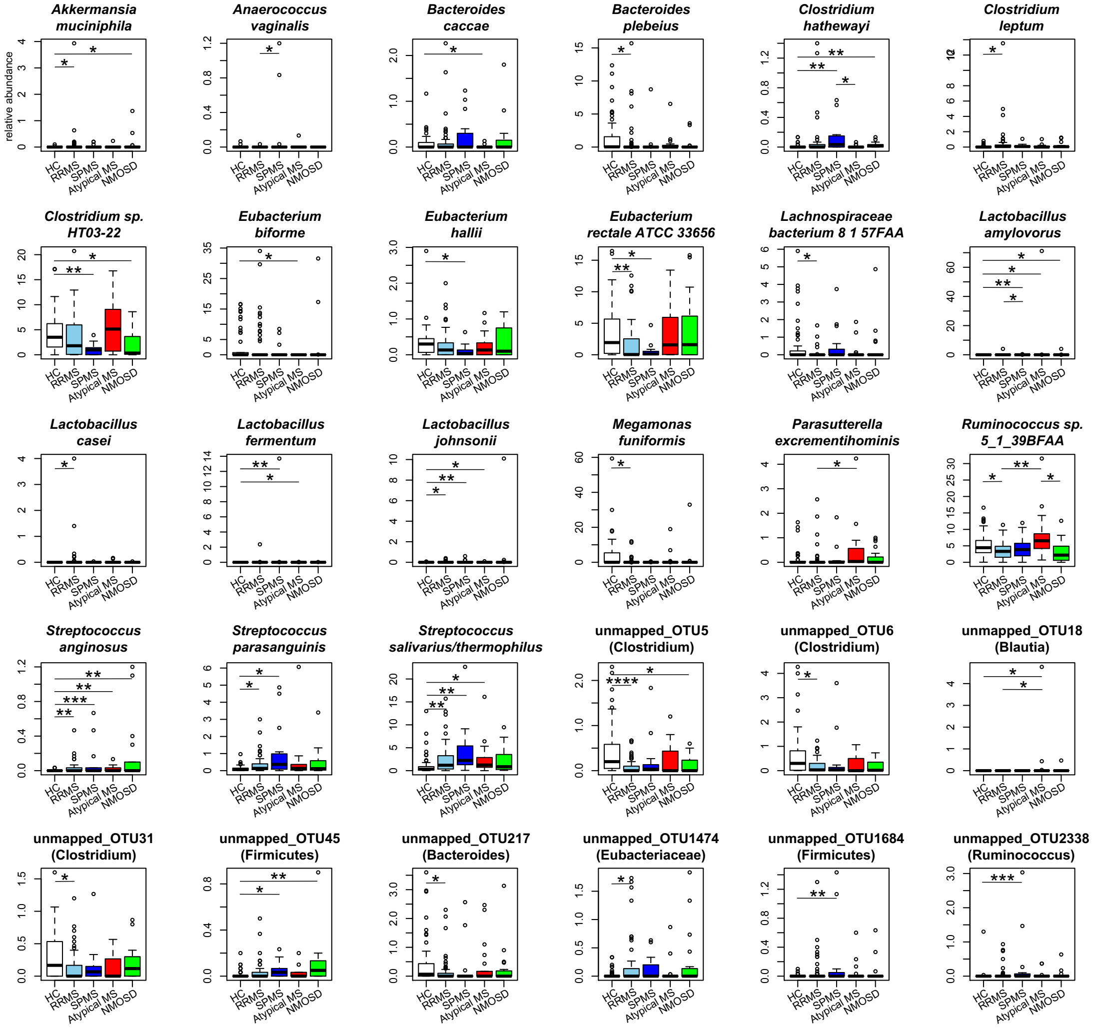
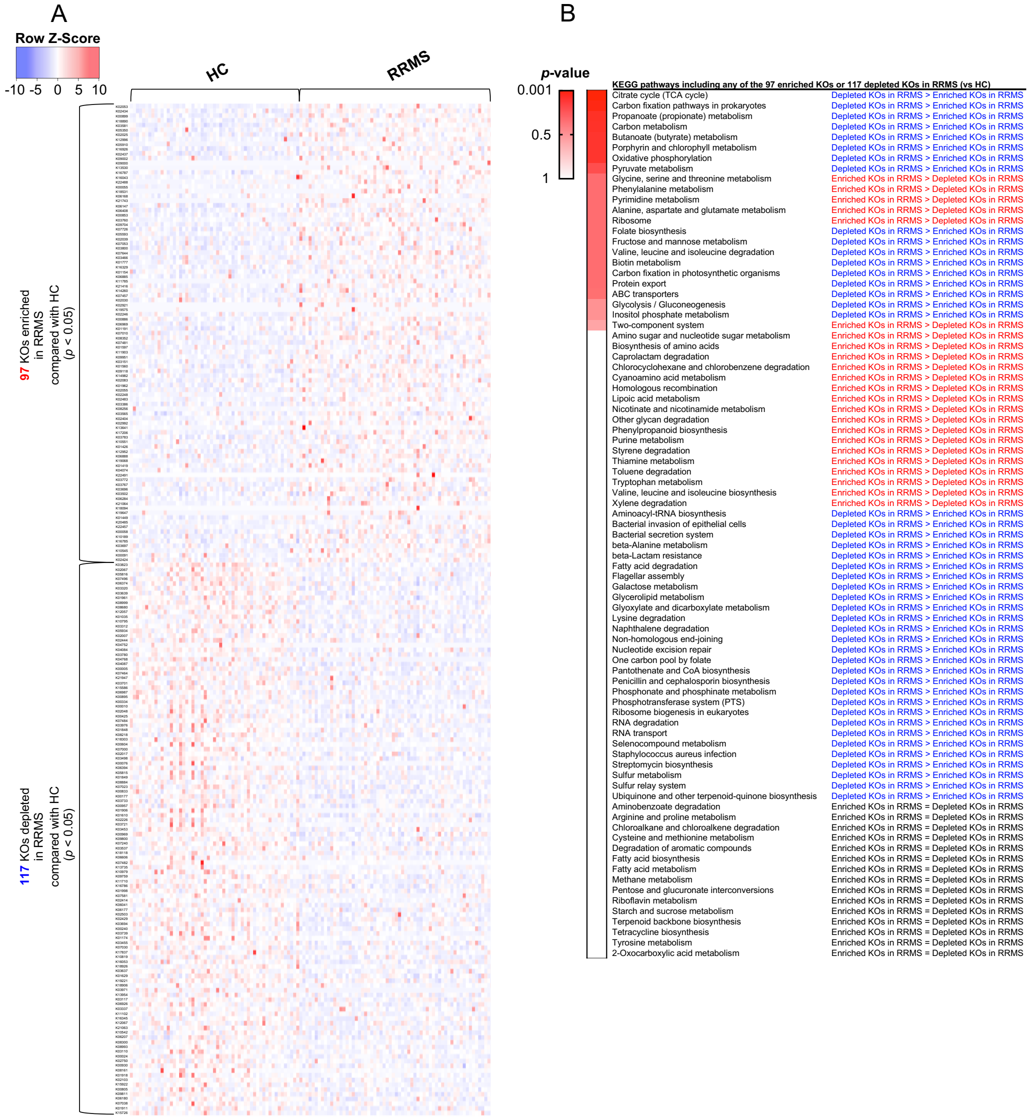

Akkermansia muciniphila
Anaerococcus vaginalis
Bacteroides caccae
Bacteroides plebeius
Clostridium hathewayi
Clostridium leptum
Clostridium sp. HT03-22
Eubacterium biforme
Eubacterium hallii
Eubacterium rectale ATCC 33656
Lachnospiraceae bacterium 8 1 57FAA
Lactobacillus amylovorus
Lactobacillus casei
Lactobacillus fermentum
Lactobacillus johnsonii
Megamonas funiformis
Parasutterella excrementihominis
Ruminococcus sp. 5_1_39BFAA
Streptococcus anginosus
Streptococcus parasanguinis
Streptococcus salivarius/thermophilus
unmapped_OTU5 (Clostridium)
unmapped_OTU6 (Clostridium)
unmapped_OTU18 (Blautia)
unmapped_OTU31 (Clostridium)
unmapped_OTU45 (Firmicutes)
unmapped_OTU217 (Bacteroides)
unmapped_OTU1474 (Eubacteriaceae)
unmapped_OTU1684 (Firmicutes)
unmapped_OTU2338 (Ruminococcus)
relative abundance
HC
RRMS
SPMS
Atypical MS
NMOSD

Figure S3



KEGG pathways including any of the 97 enriched KOs or 117 depleted KOs in RRMS (vs HC)	
Citrate cycle (TCA cycle)	Depleted KOs in RRMS > Enriched KOs in RRMS
Carbon fixation pathways in prokaryotes	Depleted KOs in RRMS > Enriched KOs in RRMS
Propanoate (propionate) metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Carbon metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Butanoate (butyrate) metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Porphyrin and chlorophyll metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Oxidative phosphorylation	Depleted KOs in RRMS > Enriched KOs in RRMS
Pyruvate metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Glycine, serine and threonine metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Phenylalanine metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Pyrimidine metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Alanine, aspartate and glutamate metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Ribosome	Enriched KOs in RRMS > Depleted KOs in RRMS
Folate biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Fructose and mannose metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Valine, leucine and isoleucine degradation	Depleted KOs in RRMS > Enriched KOs in RRMS
Biotin metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Carbon fixation in photosynthetic organisms	Depleted KOs in RRMS > Enriched KOs in RRMS
Protein export	Depleted KOs in RRMS > Enriched KOs in RRMS
ABC transporters	Depleted KOs in RRMS > Enriched KOs in RRMS
Glycolysis / Gluconeogenesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Inositol phosphate metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Two-component system	Enriched KOs in RRMS > Depleted KOs in RRMS
Amino sugar and nucleotide sugar metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Biosynthesis of amino acids	Enriched KOs in RRMS > Depleted KOs in RRMS
Caprolactam degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Chlorocyclohexane and chlorobenzene degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Cyanoamino acid metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Homologous recombination	Enriched KOs in RRMS > Depleted KOs in RRMS
Lipoic acid metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Nicotinate and nicotinamide metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Other glycan degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Phenylpropanoid biosynthesis	Enriched KOs in RRMS > Depleted KOs in RRMS
Purine metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Styrene degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Thiamine metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Toluene degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Tryptophan metabolism	Enriched KOs in RRMS > Depleted KOs in RRMS
Valine, leucine and isoleucine biosynthesis	Enriched KOs in RRMS > Depleted KOs in RRMS
Xylene degradation	Enriched KOs in RRMS > Depleted KOs in RRMS
Aminoacyl-tRNA biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Bacterial invasion of epithelial cells	Depleted KOs in RRMS > Enriched KOs in RRMS
Bacterial secretion system	Depleted KOs in RRMS > Enriched KOs in RRMS
beta-Alanine metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
beta-Lactam resistance	Depleted KOs in RRMS > Enriched KOs in RRMS
Fatty acid degradation	Depleted KOs in RRMS > Enriched KOs in RRMS
Flagellar assembly	Depleted KOs in RRMS > Enriched KOs in RRMS
Galactose metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Glycerolipid metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Glyoxylate and dicarboxylate metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Lysine degradation	Depleted KOs in RRMS > Enriched KOs in RRMS
Naphthalene degradation	Depleted KOs in RRMS > Enriched KOs in RRMS
Non-homologous end-joining	Depleted KOs in RRMS > Enriched KOs in RRMS
Nucleotide excision repair	Depleted KOs in RRMS > Enriched KOs in RRMS
One carbon pool by folate	Depleted KOs in RRMS > Enriched KOs in RRMS
Pantothenate and CoA biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Penicillin and cephalosporin biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Phosphonate and phosphinate metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Phosphotransferase system (PTS)	Depleted KOs in RRMS > Enriched KOs in RRMS
Ribosome biogenesis in eukaryotes	Depleted KOs in RRMS > Enriched KOs in RRMS
RNA degradation	Depleted KOs in RRMS > Enriched KOs in RRMS
RNA transport	Depleted KOs in RRMS > Enriched KOs in RRMS
Selenocompound metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Staphylococcus aureus infection	Depleted KOs in RRMS > Enriched KOs in RRMS
Streptomycin biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Sulfur metabolism	Depleted KOs in RRMS > Enriched KOs in RRMS
Sulfur relay system	Depleted KOs in RRMS > Enriched KOs in RRMS
Ubiquinone and other terpenoid-quinone biosynthesis	Depleted KOs in RRMS > Enriched KOs in RRMS
Aminobenzoate degradation	Enriched KOs in RRMS = Depleted KOs in RRMS
Arginine and proline metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Chloroalkane and chloroalkene degradation	Enriched KOs in RRMS = Depleted KOs in RRMS
Cysteine and methionine metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Degradation of aromatic compounds	Enriched KOs in RRMS = Depleted KOs in RRMS
Fatty acid biosynthesis	Enriched KOs in RRMS = Depleted KOs in RRMS
Fatty acid metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Methane metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Pentose and glucuronate interconversions	Enriched KOs in RRMS = Depleted KOs in RRMS
Riboflavin metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Starch and sucrose metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
Terpenoid backbone biosynthesis	Enriched KOs in RRMS = Depleted KOs in RRMS
Tetracycline biosynthesis	Enriched KOs in RRMS = Depleted KOs in RRMS
Tyrosine metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS
2-Oxocarboxylic acid metabolism	Enriched KOs in RRMS = Depleted KOs in RRMS

Table S1

	HC (n = 55)	RRMS (n = 62)	SPMS (n = 15)	Atypical MS (n = 21)	NMOSD (n = 20)	*p*-value
Age, year	40.0 ± 1.09	39.0 ± 1.00	43.3 ± 2.60	42.3 ± 1.82	43.1 ± 2.59	0.20
Sex (female : male)	19 : 36	46 : 16	9 : 6	15 : 6	18 : 2	<0.0001 / 0.23 A
BMI, kg/m^2	22.2 ± 0.30	22.0 ± 0.41	21.3 ± 0.69	22.2 ± 0.88	22.9 ± 1.03	0.46
Onset age, year		30.0 ± 1.08	27.3 ± 2.25	34.7 ± 2.00	31.9 ± 2.53	0.08
Disease duration, year		9.0 ± 0.84	15.9 ± 1.54	7.6 ± 1.02	11.2 ± 1.77	0.002 / 0.40 B
ARR		0.68 ± 0.11	0.13 ± 0.13	1.05 ± 0.25	0.95 ± 0.22	0.02
EDSS		1.88 ± 0.17	5.53 ± 0.45	2.24 ± 0.32	3.55 ± 0.42	<0.0001
Immunotherapy						
Oral PSL, %		40 (n = 25)	60 (n = 9)	67 (n = 14)	95 (n = 19)	
IS, %		5 (n = 3)	27 (n = 4)	24 (n = 5)	45 (n = 9)	
DMDs						
IFN-β, %		37 (n = 23)	13 (n = 2)	14 (n = 3)	0	
GA, %		5 (n = 3)	0	0	0	
FTY, %		6 (n = 4)	13 (n = 2)	5 (n = 1)	0	
NTZ, %		0	7 (n = 1)	0	0	
DMF, %		3 (n = 2)	0	0	0	

Table S2

Category	**No. subject**	**Weighted UniFrac**		**Unweighted UniFrac**	
		R^2	***p*-value**	**R^2**	***p*-value**
HC (female) vs HC (male)	Female: 19	0.02	0.40	0.02	0.07
	Male: 36				
RRMS receiving immunotherapy vs RRMS not receiving immunotherapy	Receiving immunotherapy: 50	0.02	0.46	0.02	0.28
	Not receiving immunotherapy: 12				

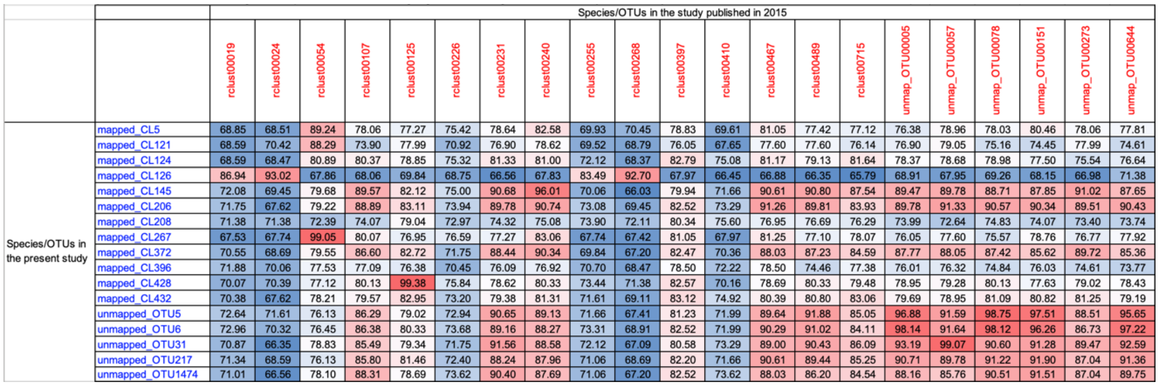
Table S3

		Average relative abundance based on 16S data					
Phylum	**Genus**	**HC**	**RRMS**	**SPMS**	**Atypical MS**	**NMOSD**	**Average**
Bacteroidetes	*Alistipes*	0.22 ± 0.03	0.80 ± 0.25	0.67 ± 0.17	0.51 ± 0.19	0.79 ± 0.24	0.6
Firmicutes	*Anaerostipes*	2.23 ± 0.35	2.65 ± 0.41	3.35 ± 1.23	2.66 ± 0.58	1.57 ± 0.41	2.49
Bacteroidetes	*Bacteroides*	14.26 ± 1.44	11.64 ± 1.25	13.82 ± 3.20	12.16 ± 2.39	10.04 ± 1.41	12.38
Actinobacteria	*Bifidobacterium*	8.07 ± 1.22	13.02 ± 1.46	13.46 ± 3.39	9.95 ± 2.32	12.49 ± 2.74	11.4
Firmicutes	*Blautia*	7.33 ± 0.69	6.96 ± 0.72	8.17 ± 2.14	7.53 ± 1.21	7.35 ± 1.31	7.47
Firmicutes	*Catenibacterium*	0.95 ± 0.42	1.27 ± 0.66	1.16 ± 1.16	0	0.07 ± 0.07	0.69
Firmicutes	*Clostridium*	12.96 ± 0.62	12.61 ± 0.81	9.86 ± 1.13	14.21 ± 1.74	12.71 ± 1.79	12.47
Actinobacteria	*Collinsella*	2.73 ± 0.33	3.23 ± 0.55	3.47 ± 0.97	2.60 ± 0.74	3.05 ± 0.74	3.01
Firmicutes	*Coprococcus*	0.44 ± 0.12	0.52 ± 0.09	0.71 ± 0.26	0.27 ± 0.08	0.75 ± 0.23	0.54
Firmicutes	*Dorea*	0.56 ± 0.06	0.58 ± 0.06	0.82 ± 0.16	0.59 ± 0.11	0.56 ± 0.11	0.62
Firmicutes	*Eubacterium*	8.32 ± 0.97	7.08 ± 0.94	3.68 ± 0.77	5.83 ± 1.17	9.50 ± 2.09	6.88
Firmicutes	*Faecalibacterium*	7.05 ± 0.66	8.66 ± 0.88	4.52 ± 1.37	7.24 ± 1.13	5.97 ± 1.24	6.69
Firmicutes	*Lactobacillus*	0.16 ± 0.08	0.46 ± 0.21	1.12 ± 0.94	3.99 ± 3.88	1.91 ± 1.19	1.53
Firmicutes	*Megamonas*	4.18 ± 1.29	0.53 ± 0.27	0.04 ± 0.03	1.28 ± 0.94	1.72 ± 1.65	1.55
Firmicutes	*Oscillibacter*	0.44 ± 0.11	0.84 ± 0.14	0.72 ± 0.20	0.73 ± 0.27	0.69 ± 0.17	0.68
Bacteroidetes	*Parabacteroides*	2.11 ± 0.30	1.49 ± 0.20	2.36 ± 0.54	2.17 ± 0.73	1.72 ± 0.53	1.97
Firmicutes	*Phascolarctobacterium*	0.67 ± 0.09	0.83 ± 0.16	0.57 ± 0.21	0.50 ± 0.13	0.60 ± 0.17	0.63
Bacteroidetes	*Prevotella*	5.44 ± 1.49	1.70 ± 0.71	1.47 ± 1.12	0.88 ± 0.64	1.59 ± 1.10	2.22
Firmicutes	*Robinsoniella*	0.32 ± 0.09	0.46 ± 0.16	2.35 ± 1.23	0.57 ± 0.35	0.58 ± 0.26	0.86
Firmicutes	*Roseburia*	2.19 ± 0.29	1.62 ± 0.24	0.75 ± 0.18	1.70 ± 0.34	1.69 ± 0.40	1.59
Firmicutes	*Ruminococcus*	11.23 ± 0.81	11.37 ± 0.92	11.54 ± 2.11	13.56 ± 1.64	11.85 ± 1.69	11.91
Firmicutes	*Streptococcus*	2.30 ± 0.52	4.10 ± 0.64	6.40 ± 1.89	6.07 ± 2.26	3.67 ± 0.98	4.51
Firmicutes	*Subdoligranulum*	0.42 ± 0.11	0.86 ± 0.21	0.50 ± 0.20	0.13 ± 0.05	0.20 ± 0.09	0.42

Table S4

Species/OTU ID	Closest species	Identity (%) 1	Group 1 (G1)	increase or decrease	Group 2 (G2)	LOG10 (G1 / G2) 2	Average relative abundance in G2 (%)	Adjusted *p*-value (G1 vs G2)
mapped_CL5	*Streptococcus parasanguinis*	-	RRMS	>	HC	0.43	0.12	0.03
mapped_CL5	*Streptococcus parasanguinis*	-	SPMS	>	HC	0.93	0.12	0.03
mapped_CL9	*Anaerococcus vaginalis*	-	SPMS	>	RRMS	2.41	0.0005	0.04
mapped_CL14	*Bacteroides caccae*	-	Atypical MS	<	HC	-0.96	0.09	0.03
mapped_CL78	*Eubacterium hallii*	-	SPMS	<	HC	-0.49	0.34	0.04
mapped_CL121	*Streptococcus anginosus*	-	RRMS	>	HC	1.13	0.002	0.01
mapped_CL121	*Streptococcus anginosus*	-	SPMS	>	HC	1.72	0.002	0.0004
mapped_CL121	*Streptococcus anginosus*	-	Atypical MS	>	HC	1.05	0.002	0.002
mapped_CL121	*Streptococcus anginosus*	-	NMOISD	>	HC	1.95	0.002	0.004
mapped_CL124	*Lactobacillus casei*	-	RRMS	>	HC	0.49	0	0.03
mapped_CL126	*Bacteroides plebeius*	-	RRMS	<	HC	-0.30	1.48	0.01
mapped_CL129	*Lactobacillus fermentum*	-	SPMS	>	HC	1.44	0	0.008
mapped_CL129	*Lactobacillus fermentum*	-	Atypical MS	>	HC	-0.85	0	0.04
mapped_CL145	*Eubacterium rectale ATCC 33656*	-	RRMS	<	HC	-0.33	3.60	0.005
mapped_CL145	*Eubacterium rectale ATCC 33656*	-	SPMS	<	HC	-0.79	3.60	0.03
mapped_CL169	*Clostridium sp. HT03-22*	-	SPMS	<	HC	-0.63	4.41	0.001
mapped_CL169	*Clostridium sp. HT03-22*	-	NMOISD	<	HC	-0.34	4.41	0.04
mapped_CL206	*Lachnospiraceae bacterium 8 1 57FAA*	-	RRMS	<	HC	-0.94	0.53	0.01
mapped_CL208	*Akkermansia muciniphila*	-	RRMS	>	HC	1.59	0.002	0.03
mapped_CL208	*Akkermansia muciniphila*	-	NMOISD	>	HC	1.63	0.002	0.04
mapped_CL267	*Streptococcus salivarius/thermophilus*	-	RRMS	>	HC	0.34	1.16	0.004
mapped_CL267	*Streptococcus salivarius/thermophilus*	-	SPMS	>	HC	0.57	1.16	0.002
mapped_CL267	*Streptococcus salivarius/thermophilus*	-	Atypical MS	>	HC	0.33	1.16	0.02
mapped_CL271	*Lactobacillus amylovorus*	-	SPMS	>	HC	0.05	0	0.008
mapped_CL271	*Lactobacillus amylovorus*	-	Atypical MS	>	HC	2.01	0	0.04
mapped_CL271	*Lactobacillus amylovorus*	-	NMOISD	>	HC	0.79	0	0.03
mapped_CL271	*Lactobacillus amylovorus*	-	SPMS	<	RRMS	-0.24	0.07	0.04
mapped_CL372	*Ruminococcus sp. 5_1_39BFAA*	-	RRMS	<	HC	-0.17	5.24	0.04
mapped_CL372	*Ruminococcus sp. 5_1_39BFAA*	-	RRMS	<	Atypical MS	-0.35	7.78	0.01
mapped_CL372	*Ruminococcus sp. 5_1_39BFAA*	-	Atypical MS	>	NMOSD	0.38	3.24	0.02
mapped_CL396	*Lactobacillus johnsonii*	-	RRMS	>	HC	0.93	0.003	0.03
mapped_CL396	*Lactobacillus johnsonii*	-	SPMS	>	HC	1.41	0.003	0.003
mapped_CL396	*Lactobacillus johnsonii*	-	Atypical MS	>	HC	0.76	0.003	0.04
mapped_CL406	*Parasutterella excrementihominis*	-	RRMS	<	Atypical MS	-0.58	0.44	0.02
mapped_CL428	*Megamonas funiformis*	-	RRMS	<	HC	-0.89	4.17	0.01
mapped_CL429	*Eubacterium biforme*	-	Atypical MS	<	HC	-1.90	2.64	0.01
mapped_CL432	*Clostridium leptum*	-	RRMS	>	HC	0.98	0.06	0.02
mapped_CL446	*Clostridium hathewayi*	-	SPMS	>	HC	1.16	0.01	0.001
mapped_CL446	*Clostridium hathewayi*	-	NMOISD	>	HC	0.50	0.01	0.006
mapped_CL446	*Clostridium hathewayi*	-	SPMS	>	Atypical MS	1.02	0.01	0.03
unmapped_OTU5	*Clostridium indolis*	95.34	RRMS	<	HC	-0.65	0.44	0.00002
unmapped_OTU5	*Clostridium indolis*	95.34	NMOISD	<	HC	-0.55	0.44	0.04
unmapped_OTU6	*Clostridium indolis*	95.99	RRMS	<	HC	-0.52	0.65	0.01
unmapped_OTU18	*Blautia luti*	94.12	Atypical MS	>	HC	0.87	0	0.04
unmapped_OTU18	*Blautia luti*	94.12	RRMS	<	Atypical MS	-0.87	0.25	0.03
unmapped_OTU31	*Clostridium symbiosum*	93.83	RRMS	<	HC	-0.38	0.31	0.03
unmapped_OTU45	*Clostridiales 303A04*	89.16	SPMS	>	HC	0.56	0.02	0.04
unmapped_OTU45	*Clostridiales 303A04*	89.16	NMOISD	>	HC	0.84	0.02	0.002
unmapped_OTU217	*Bacteroides xylanolyticus*	94.75	RRMS	<	HC	-0.40	0.44	0.04
unmapped_OTU1474	*Eubacterium fissicatena*	93.56	RRMS	>	HC	0.80	0.03	0.02
unmapped_OTU1684	*Anaerovorax odorimutans*	87.31	SPMS	>	HC	1.59	0.005	0.005
unmapped_OTU2338	*Ruminococcus obeum*	95.98	SPMS	>	HC	1.13	0.02	0.0009

Table S5



		Species/OTUs in the study published in 2015																				
		rclust00019	rclust00024	rclust00054	rclust00107	rclust00125	rclust00226	rclust00231	rclust00240	rclust00255	rclust00268	rclust00397	rclust00410	rclust00467	rclust00489	rclust00715	unmap_OTU00005	unmap_OTU00057	unmap_OTU00078	unmap_OTU00151	unmap_OTU00273	unmap_OTU00644
Species/OTUs in the present study	mapped_CL5	68.85	68.51	89.24	78.06	77.27	75.42	78.64	82.58	69.93	70.45	78.83	69.61	81.05	77.42	77.12	76.38	78.96	78.03	80.46	78.06	77.81
	mapped_CL121	68.59	70.42	88.29	73.90	77.99	70.92	76.90	78.62	69.52	68.79	76.05	67.65	77.60	77.60	76.14	76.90	79.05	75.16	74.45	77.99	74.61
	mapped_CL124	68.59	68.47	80.89	80.37	78.85	75.32	81.33	81.00	72.12	68.37	82.79	75.08	81.17	79.13	81.64	78.37	78.68	78.98	77.50	75.54	76.64
	mapped_CL126	86.94	93.02	67.86	68.06	69.84	68.75	66.56	67.83	83.49	92.70	67.97	66.45	66.88	66.35	65.79	68.91	67.95	69.26	68.15	66.98	71.38
	mapped_CL145	72.08	69.45	79.68	89.57	82.12	75.00	90.68	96.01	70.06	66.03	79.94	71.66	90.61	90.80	87.54	89.47	89.78	88.71	87.85	91.02	87.65
	mapped_CL206	71.75	67.62	79.22	88.89	83.11	73.94	89.78	90.74	73.08	69.45	82.52	73.29	91.26	89.81	83.93	89.78	91.33	90.57	90.34	89.51	90.43
	mapped_CL208	71.38	71.38	72.39	74.07	79.04	72.97	74.32	75.08	73.90	72.11	80.34	75.60	76.95	76.69	76.29	73.99	72.64	74.83	74.07	73.40	73.74
	mapped_CL267	67.53	67.74	99.05	80.07	76.95	76.59	77.27	83.06	67.74	67.42	81.05	67.97	81.25	77.10	78.07	76.05	77.60	75.57	78.76	76.77	77.92
	mapped_CL372	70.55	68.69	79.55	86.60	82.72	71.75	88.44	90.34	69.84	67.20	82.47	70.36	88.03	87.23	84.59	87.77	88.05	87.42	85.62	89.72	85.36
	mapped_CL396	71.88	70.06	77.53	77.09	76.38	70.45	76.09	76.92	70.70	68.47	78.50	72.22	78.50	74.46	77.38	76.01	76.32	74.84	76.03	74.61	73.77
	mapped_CL428	70.07	70.39	77.12	80.13	99.38	75.84	78.62	80.33	73.44	71.38	82.57	70.16	78.69	80.33	79.48	78.95	79.28	80.13	77.63	79.02	78.43
	mapped_CL432	70.38	67.62	78.21	79.57	82.95	73.20	79.38	81.31	71.61	69.11	83.12	74.92	80.39	80.80	83.06	79.69	78.95	81.09	80.82	81.25	79.19
	unmapped_OTU5	72.64	71.61	76.13	86.29	79.02	72.94	90.65	89.13	71.66	67.41	81.23	71.99	89.64	91.88	85.05	96.88	91.59	98.75	97.51	88.51	95.65
	unmapped_OTU6	72.96	70.32	76.45	86.38	80.33	73.68	89.16	88.27	73.31	68.91	82.52	71.99	90.29	91.02	84.11	98.14	91.64	98.12	96.26	86.73	97.22
	unmapped_OTU31	70.87	66.35	78.83	85.49	79.34	71.75	91.56	88.58	72.12	67.09	80.58	73.29	89.00	90.43	86.09	93.19	99.07	90.60	91.28	89.47	92.59
	unmapped_OTU217	71.34	68.59	76.13	85.80	81.46	72.40	88.24	87.96	71.06	68.69	82.20	71.66	90.61	89.44	85.25	90.71	89.78	91.22	91.90	87.04	91.36
	unmapped_OTU1474	71.01	66.56	78.10	88.31	78.69	73.62	90.40	87.69	71.06	67.20	82.52	73.62	88.03	86.20	84.54	88.16	85.76	90.51	91.51	87.04	89.75

Table S6

Group 1 (G1)	Group 2 (G2)	KOs with significant changes in abundance	Significantly enriched KOs in G1 compared with G2	Significantly depleted KOs in G1 compared with G2	Number of KEGG pathways including at least one of the significantly enriched KOs or depleted KOs in G1 compared with G2
RRMS	**HC**	214	97	117	83
SPMS	**HC**	144	89	55	86
Atypical MS	**HC**	65	45	20	46
NMOSD	**HC**	149	105	44	80
SPMS	**RRMS**	52	38	14	38
RRMS	**Atypical MS**	48	10	38	33
RRMS	**NMOSD**	39	4	35	29
SPMS	**Atypical MS**	44	23	21	32
SPMS	**NMOSD**	29	18	11	29
Atypical MS	**NMOSD**	55	21	34	44

Table S7

	HC (n = 8)	RRMS (n = 12)	SPMS (n = 9)	*p*-value
Age, year	36.5 ± 2.24	39.0 ± 2.02	38.7 ± 1.56	0.64
Sex (female : male)	4 : 4	7 : 5	5 : 4	0.93
BMI, kg/m^2	21.8 ± 0.79	22.6 ± 1.20	21.3 ± 1.07	0.58
Onset age, year		28.5 ± 2.19	24.0 ± 1.43	0.14
Disease duration, year		10.5 ± 2.54	14.7 ± 2.12	0.13
ARR		0.67 ± 0.26	0.22 ± 0.22	0.21
EDSS		1.83 ± 0.27	5.39 ± 0.55	<0.0001
Immunotherapy, %		92 (n = 11)	89 (n = 8)	>0.99