Supplementary method

1. Experiment procedure

1.1 DNA extraction and PCR amplification

Microbial DNA was extracted using the HiPure Soil DNA Kits (or HiPure Stool DNA Kits) (Magen, Guangzhou, China) according to manufacturer's protocols. The 16S rDNA target (listed in the table) region of the ribosomal RNA gene were amplified by PCR (94°C for 2 min, followed by 30 cycles at 98°C for 10 s, 62°C for 30 s (except for 16S V4: 55°C for 30 s), and 68°C for 30 s and a final extension at 68°C for 5 min） using primers listed in the table[1]. PCR reactions were performed in triplicate 50 μL mixture containing 5 μL of 10 × KOD Buffer, 5 μL of 2 mM dNTPs, 3 μL of 25 mM $MgSO_4$, 1.5 μL of each primer (10 μM), 1 μL of KOD Polymerase, and 100 ng of template DNA. Related PCR reagents were from TOYOBO，Japan. Other primers are listed in Table 1.

Table 1. primer information

type	region	primer name	primer sequence	product length	reference
16S	V4	515F	GTGYCAGCMGCCGCGGTAA	~292	40,41
		806R	GGACTACNVGGGTWTCTAAT		
16S	V3-V4	341F	CCTACGGGNGGCWGCAG	~466	1
		806R	GGACTACHVGGGTATCTAAT		
16S	V4-V5	515F	GTGCCAGCMGCCGCGGTAA	~412	2
		907R	CCGTCAATTCCTTTGAGTTT		
16S	V5-V7	799F	AACMGGATTAGATACCCKG	~414	3
		1193R	ACGTCATCCCCACCTTCC		

16S	V4-V5	Arch519F Arch915R	CAGCMGCCGCGGTAA GTGCTCCCCCGCCAATTCCT	~416	4
18S	V4	528F 706R	GCGGTAATTCCAGCTCCAA AATCCRAGAATTTCACCTCT	~260	5
ITS	ITS1	ITS1_F_KYO2 ITS86R	TAGAGGAAGTAAAAGTCGTAA TTCAAAGATTCGATGATTCAC	~366	42
ITS	ITS1	ITS1-F ITS2	CTTGGTCATTTAGAGGAAGTAA GCTGCGTTCTTCATCGATGC	~321	6
ITS	ITS2	ITS3_KYO2 ITS4	GATGAAGAACGYAGYRAA TCCTCCGCTTATTGATATGC	~381	6

1.2 Illumina Novaseq 6000 sequencing

Amplicons were extracted from 2% agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using ABI StepOnePlus Real-Time PCR System (Life Technologies，Foster City, USA). Purified amplicons were pooled in equimolar and paired-end sequenced (PE250) on an Illumina platform according to the standard protocols. The raw reads were deposited into the NCBI Sequence Read Archive (SRA) database.

2. Bioinformatics analysis

2.1 Quality control and clustering (DADA2 method or Usearch method)

2.1.1 DADA2 method

The DADA2 R package[17] (version 1.14) implements a complete pipeline to turn paired-end fastq files from the sequencer into merged, denoised, chimera-free,

inferred sample sequences. In datail：

(1) Filtering

Raw reads containing primers or unknown nucleotides (N bases) would affect the following assembly and analysis. Thus, to get clean reads, raw reads were filtered and truncated according to the following rules:

1) Removing reads containing unknown nucleotides (N);

2) Removing primer sequences.

(2) Dereplication and denoising

Then, a dereplicated list of unique sequences and their abundances were output, as well as the consensus positional quality scores for each unique sequence by taking the average (mean) of the positional qualities of the component reads. These consensus scores are used by the error model. Considering that each amplicon sequencing sample had different error ratio, DADA2 used machine learning to construct the error model for reads denoising, by alternately estimating the error rate and learning the error model from the reference sample sequence until the learning model converges to the true error rate.

(3) Merging

Then paired end denoised reads were merged as raw ASVs (amplicon sequence variants) with a minimum overlap of 12bp.

2.1.4 Chimera removal

Chimeras are identified by performing a Needleman-Wunsch global alignment of each sequence to all more abundant sequences, and then searching for combinations

of a left-parent and a right-parent that cover the child sequence without any mismatches or internal indels. After chimera removal, the denoised, chimera-free ASV sequences and their abundances were output.

2.1.2 Usearch method

(1) Reads filtering

Raw data containing adapters or low quality reads would affect the following assembly and analysis. Thus, to get high quality clean reads, raw reads were further filtered according to the following rules using FASTP[7] (version 0.18.0):

1) Removing reads containing more than 10% of unknown nucleotides (N);

2) Removing reads containing less than 50% of bases with quality (Q-value)＞20.

(2) Reads assembly

Paired end clean reads were merged as raw tags using FLSAH[8] (version 1.2.11) with a minimum overlap of 10 bp and mismatch error rates of 2%.

(3) Raw tag filtering

Noisy sequences of raw tags were filtered under specific filtering conditions[39] to obtain the high-quality clean tags. The filtering conditions are as follows：

1) Break raw tags from the first low quality base site where the number of bases in the continuous low quality value (the default quality threshold is≤3) reaches the set length (the default length is 3 bp)；

2)Then, filter tags whose continuous high-quality base length is less than 75% of the tag length.

(4) Clustering and chimera removal

The clean tags were clustered into operational taxonomic units (OTUs) of ⩾ 97 % similarity using UPARSE[11] (version 9.2.64) pipeline. All chimeric tags were removed using UCHIME algorithm[10] and finally obtained effective tags for further analysis. The tag sequence with highest abundance was selected as representative sequence within each cluster.

2.2 Community composition analysis

The representative OTU sequences or ASV sequences were classified into organisms by a naive Bayesian model using RDP classifier[15] (version 2.2) based on SILVA[16] database (version 132) or UNITE[18] database (version 8.0) or ITS2[19] database (version update_2015), with the confidence threshold value of 0.8. The abundance statistics of each taxonomy was visualized using Krona[20] (version 2.6). The stacked bar plot of the community composition was visualized in R project ggplot2 package[21](version 2.2.1). Circular layout representations of species abundance were graphed using circos[22] (version 0.69-3). Heatmap of species abundance was plotted using pheatmap package (version 1.0.12)[23] in R project. Pearson correlation analysis of species was calculated in R project psych package[37] (version 1.8.4). Network of correlation coefficient were generated using Omicsmart, a dynamic real-time interactive online platform for data analysis (http://www.omicsmart.com) or igraph package[38] (version 1.1.2) in R project.

2.3 Indicator species analysis

Between groups Venn analysis was performed in R project VennDiagram package[12] (version 1.6.16) and upset plot was performed in R project UpSetR package

[13] (version 1.3.3) to identify unique and common Species or OTUs or ASVs. Species comparison between groups was calculated by Welch's t-test and Wilcoxon rank test in R project Vegan package[14] (version 2.5.3). Species comparison among groups was computed by Tukey's HSD test and Kruskal-Wallis H test in R project Vegan package[14] (version 2.5.3). Biomarker features in each group were screened by LEfSe software[24] (version 1.0), randomforest package[25] (version 4.6.12) in R project, pROC package[26] (version 1.10.0) in R project, and labdsv package[27](version2.0-1) in R project. Ternary plot of species abundance was plotted using R ggtern package[28] (version 3.1.0).

2.4 Alpha diversity analysis

Chao1, ACE, Shannon, Simpson, Good's coverage, Pielou's evenness index were calculated in QIIME[9] (version 1.9.1). PD-whole tree index was calculated in picante[43] (version 1.8.2). OTU/ASV rarefaction curve and rank abundance curves were plotted in R project ggplot2 package[21] (version 2.2.1). Alpha index comparison between groups was calculated by Welch's t-test and Wilcoxon rank test in R project Vegan package[14] (version 2.5.3). Alpha index comparison among groups was computed by Tukey's HSD test and Kruskal-Wallis H test in R project Vegan package[14] (version 2.5.3).

2.5 Beta diversity analysis

Sequence alignment was performed using Muscle[29](version 3.8.31) and phylogenetic tree was constructed using FastTree[30](version 2.1), then weighted and unweighted unifrac distance matrix were generated by GuniFrac package[31] (version

1.0) in R project. Jaccard and bray-curtis distance matrix calculated in R project Vegan package[14](version 2.5.3). PCA (principal component analysis) was performed in R project Vegan package[14](version 2.5.3). Multivariate statistical techniques including PCoA (principal coordinates analysis) and NMDS (non-metric multi-dimensional scaling) of (Un) weighted unifrac, jaccard and bray-curtis distances were generated in R project Vegan package[14] (version 2.5.3) and plotted in R project ggplot2 package[21] (version 2.2.1). Statistic analysis of Welch's t-test, Wilcoxon rank test, Tukey's HSD test, Kruskal-Wallis H test, Adonis (also called Permanova) and Anosim test was calculated in R project Vegan package[14](version 2.5.3).

2.6 Function prediction

The KEGG pathway analysis of the OTUs/ASV was inferred using Tax4Fun[32](version 1.0) or PICRUSt[33] (version 2.1.4). Microbiome phenotypes of bacteria were classified using BugBase[34]. FAPROTAX database (Functional Annotation of Prokaryotic Taxa) and associated software[35](version 1.0) were used for generating the ecological functional profiles of bacteria. The Functional group (guild) of the Fungi was inferred using FUNGuild[36] (version 1.0). Analysis of function difference between groups was calculated by Welch's t-test, Wilcoxon rank test and Kruskal-Wallis H test, Tukey's HSD test in R project Vegan package[14] (version 2.5.3).

2.7 Environmental factor analysis

Redundancy analysis (RDA), canonical correspondence analysis (CCA), Variation partition analysis (VPA), mantel test and envfit test were executed in R

project Vegan package[14](version 2.5.3) to clarify the influence of environmental factors on community composition. Pearson correlation coefficient between environmental factors and species was calculated in R project psych package[37] (version 1.8.4). Heatmap and network of correlation coefficient were generated using Omicsmart, a dynamic real-time interactive online platform for data analysis (http://www.omicsmart.com).

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Supplementary Figure

Fig.1S

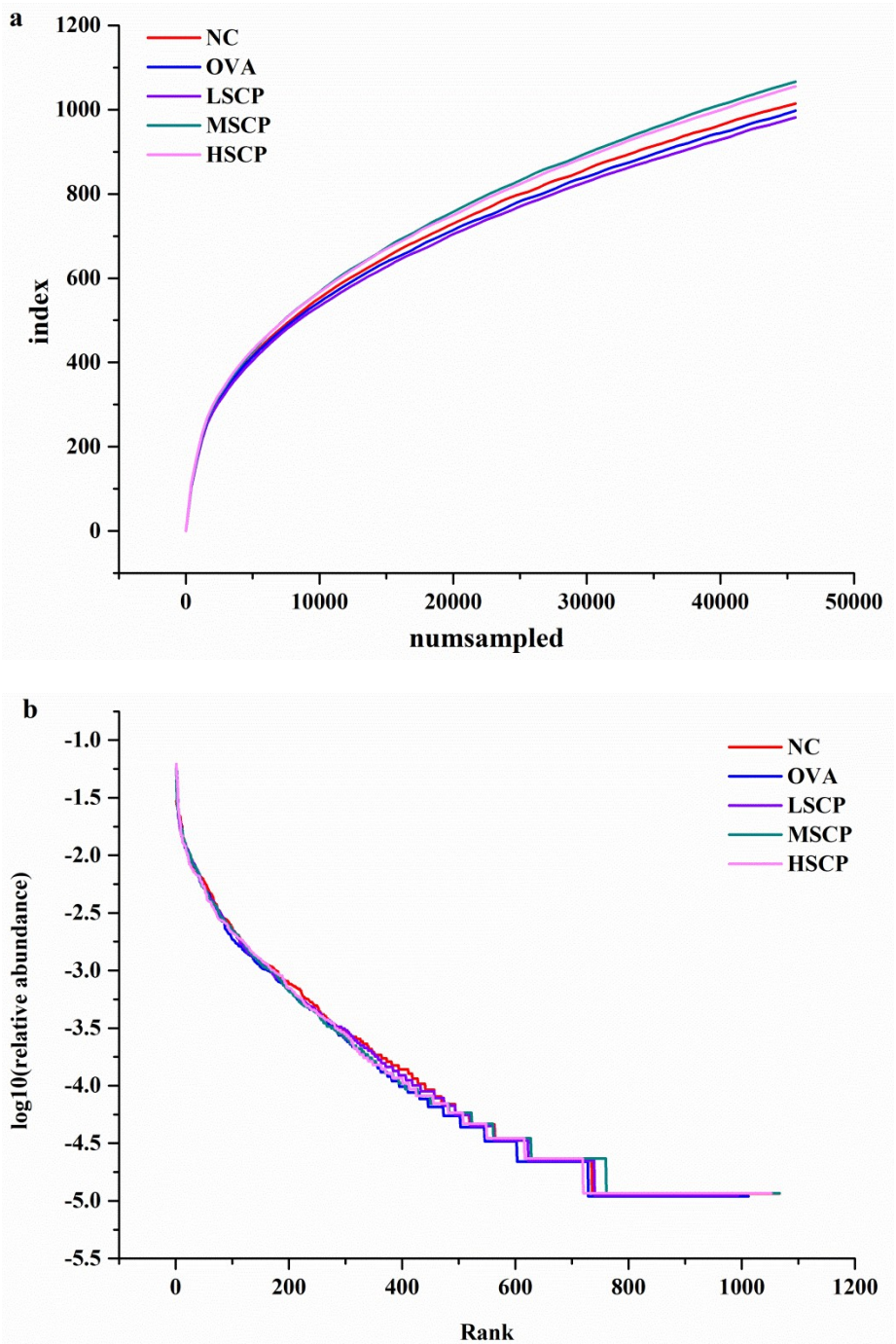

Figure S1. Gut microbiota diversity curve of different groups. a: The rarefaction curve of gut microbiota based on random extraction of sequencing data from cecal digestives. b: Rank abundance based on the relative abundance of OTUs.

Fig.2S

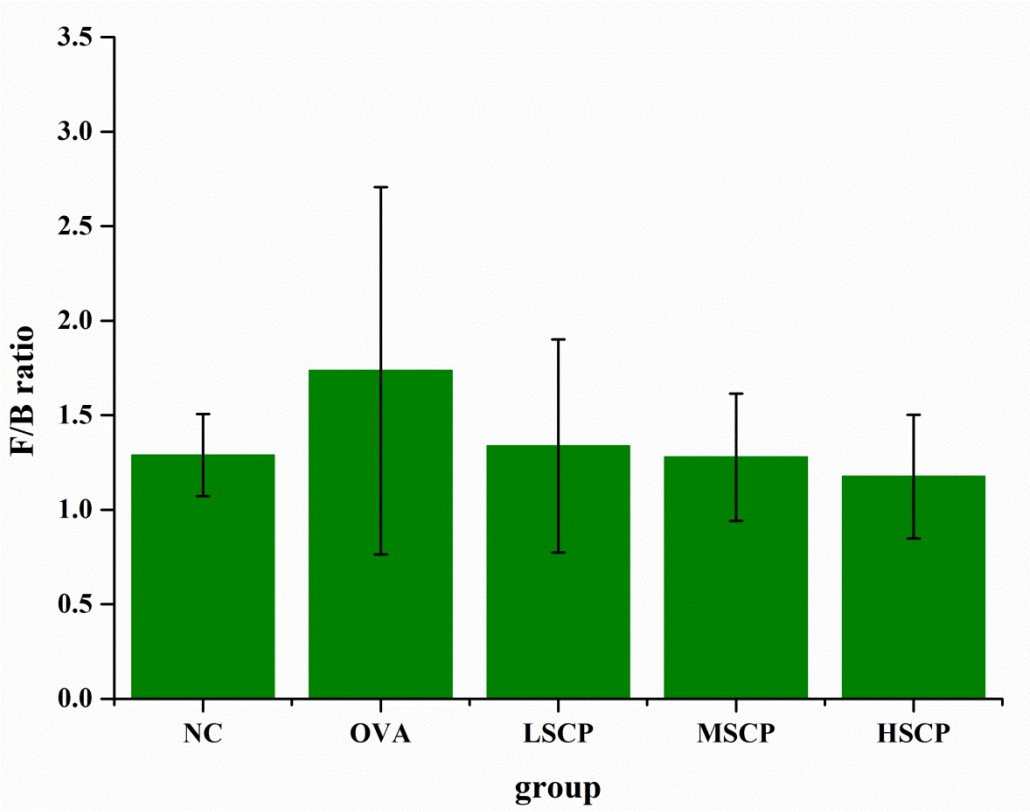

Fig.2S Firmicutes/Bacteroidetes ratio

Abbreviations: NC, normal control group; OVA, OVA-model control group; LSCP, LSCP-low-dose SCP group; MSCP, MSCP-medium-dose SCP group; HSCP, HSCP-high-dose SCP group.

Fig.3S

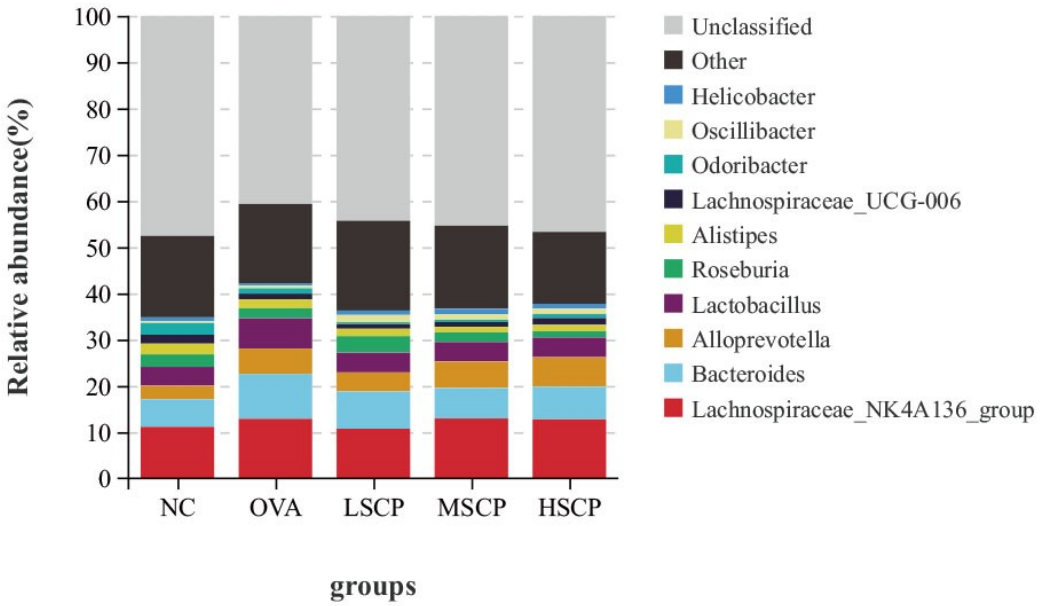

Fig.3S Relative abundance of predominant taxa at the genus level in the fecal

Table S1 Relative abundance of predominant taxa at the phylum level

Group	Firmicutes (%)	Bacteroidetes(%)	Proteobacteria (%)
NC	49.46±9.07	42.63±10.16	3.83±1.81
OVA	53.39±14.20	42.12±14.09	2.03±0.56
LSCP	49.45±17.10	44.39±14.19	2.18±0.72
MSCP	53.16±6.82	40.46±5.83	2.73±0.45
HSCP	51.71±7.95	41.50±8.10	3.64±1.60

Table S2 Relative abundance of predominant taxa at the family level

Group	Lachnospiraceae (%)	Muribaculaceae (%)	Ruminococcaceae (%)	Bacteroidaceae (%)	Prevotellaceae (%)	Lactobacillaceae (%)
NC	36.12±5.14	23.74±5.15	8.75±2.77	4.75±1.17	4.78±2.95	4.08±3.44
OVA	30.23±5.54	22.29±1.75	8.13±1.92	8.62±1.89	7.12±4.03	6.82±3.53
LSCP	38.06±3.02	17.94±3.48	12.82±0.57	7.19±3.88	3.73±1.94	4.90±2.11
MSCP	38.12±3.32	20.57±7.98	13.54±3.67	6.31±2.55	6.25±4.81	4.27±1.28
HSCP	32.11±5.92	26.30±6.25	10.57±0.64	7.25±1.43	6.20±4.47	4.18±1.07

Table S3 Relative abundance of predominant taxa at the genus level

Group	Lachnospiraceae_ NK4A136_group(%)	Bacteroides (%)	Alloprevotella (%)	Lactobacillus (%)	Roseburia (%)	Alistipes (%)	Lachnospiraceae_ UCG-006(%)	Odoribacter (%)	Oscillibacter (%)	Helicobacter (%)	Other (%)	Unclassified (%)
NC	11.14±3.99	5.97±3.19	2.89±2.69	4.08±3.08	2.72±1.90	2.30±1.59	1.93±1.02	2.55±2.12	0.36±0.35	0.84±0.83	17.64±4.80	47.57±5.67
OVA	12.88±5.90	9.70±8.23	5.46±3.82	6.56±4.24	2.18±1.03	1.92±0.55	1.24±0.99	1.16±0.99	0.54±0.26	0.54±0.49	17.15±2.40	40.70±4.80
LSCP	10.72±5.66	8.02±3.05	4.16±2.02	4.28±3.15	3.56±2.06	1.59±0.6	1.00±0.86	0.42±0.39	1.56±0.76	0.96±0.76	19.44±3.93	44.29±7.73
MSCP	12.95±6.27	6.58±3.08	5.73±3.08	4.22±2.01	2.11±0.93	1.16±0.63	1.01±0.33	0.52±0.18	1.17±0.52	1.18±1.47	17.99±3.05	45.38±5.86
HSCP	12.75±6.44	7.06±1.59	6.35±4.13	4.29±2.16	1.37±0.87	1.34±0.53	1.52±1.25	0.88±0.74	1.10±0.47	1.09±0.89	15.54±2.14	46.72±6.11