

Airway Microbiome in Different Inflammatory Phenotypes of Asthma:

A Cross-sectional Study in Northeast China

Zhiqiang Pang¹, Guoqiang Wang¹, Peter Gibson², Xuewa Guan¹, Weijie Zhang³, Ruipeng Zheng^{1,4}, Fang Chen¹, Ziyang Wang¹ and Fang Wang^{1*}

1. Department of Pathogen Biology, College of Basic Medical Sciences, Jilin University, Changchun, China; 2. Department of Respiratory and Sleep Medicine, John Hunter Hospital, Newcastle, Australia; 3. Third Department of Respiratory Disease, Jilin Provincial People's Hospital, Changchun, China; 4. Department of Interventional Therapy, Bethune First Hospital, Jilin University, Changchun, China;

Corresponding author: Fang Wang*, Department of Pathogen Biology, College of Basic Medical Sciences, Jilin University, NO. 828 of Xinmin Street, Changchun, China, Email: wf@jlu.edu.cn

Figure S1- Numbers of OTUs in all samples in different taxonomic levels.

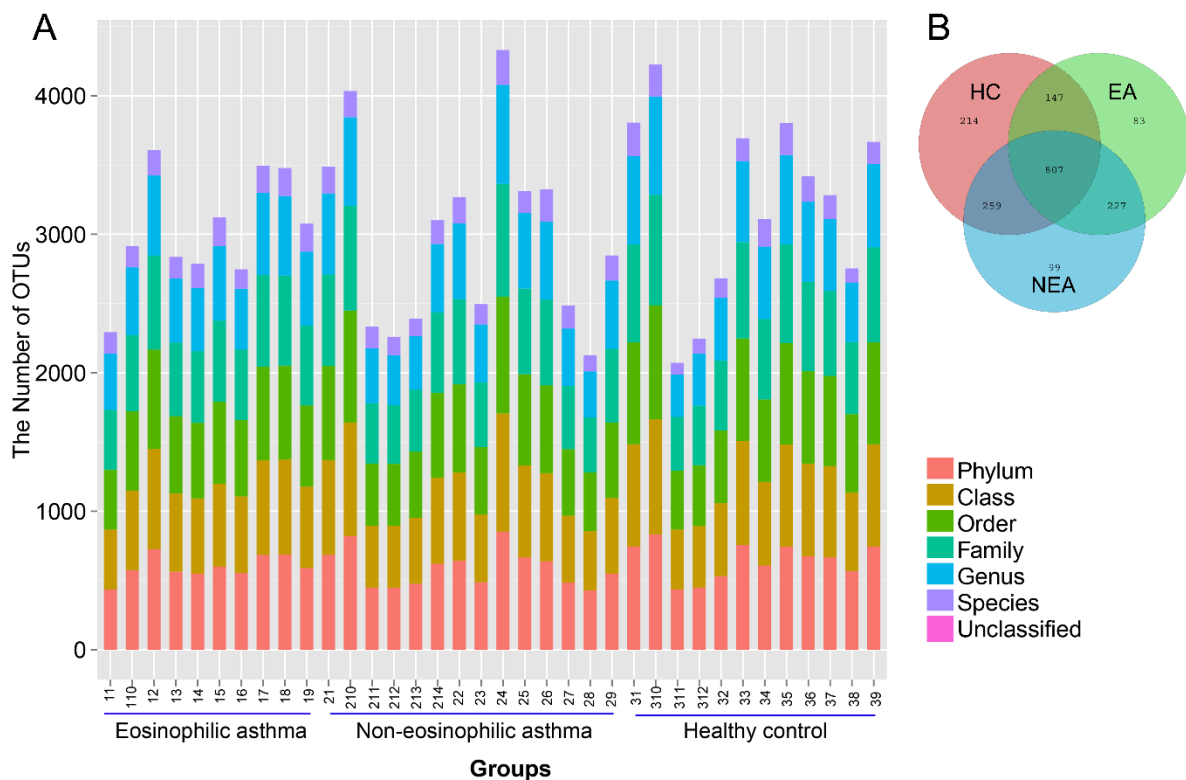


Figure S1 Numbers of OTUs in all samples in different taxonomic levels. **(A)** All OTUs had been identified into the respective taxonomic level. No OUT was unclassified in all samples. **(B)** Venn diagram of all identified OTUs in the three groups. The overlapped part of the circles represents the shared OTUs.

Figure S2- Species accumulation curve of all samples

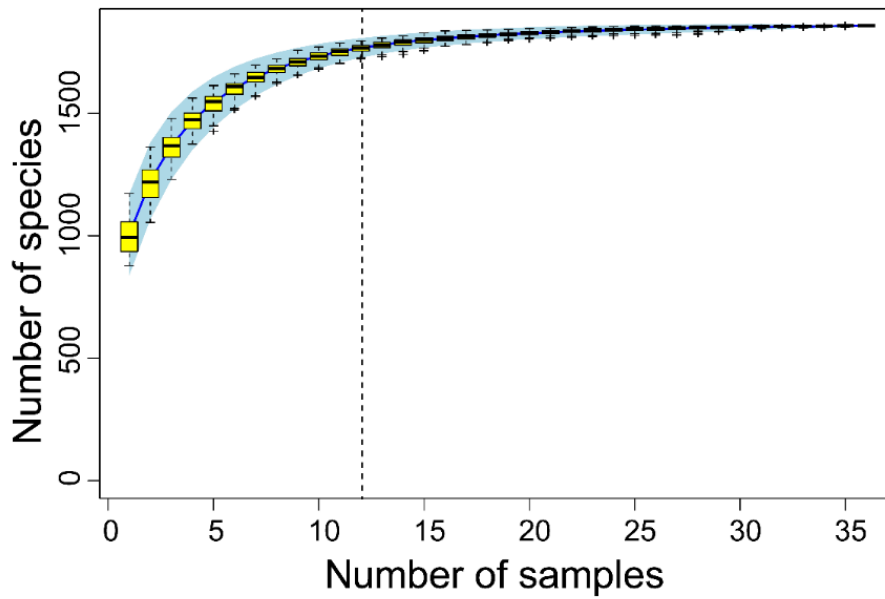


Figure S2 Numbers of species observed after the increase of samples included. The plateau of the curve indicated an appropriate sample number included for study.

Figure S3 Evaluation on Pielou Evenness significance analysis in the groups.

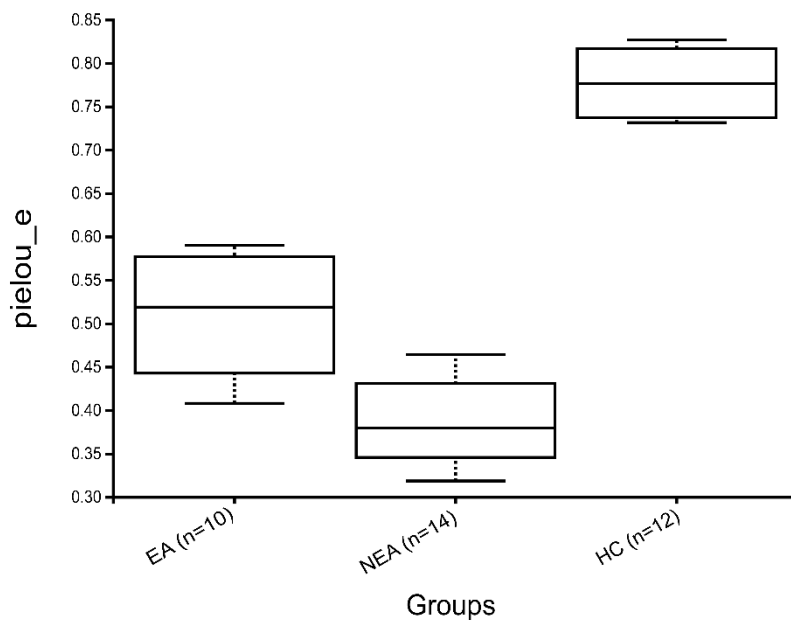


Figure S3 Evaluation on Pielou Evenness significance in the groups. There is a significant difference among the three groups (EA vs NEA, $P < 0.05$, EA vs HC & NEA vs HC, $P < 0.01$).

Table S1 Kruskal-Wallis (Pairwise) Results of the Pielou Evenness Analysis

Group1	Group2	H	<i>P-value</i>	<i>q-value</i>
EA (n=10)	NEA (n=14)	3.8846	0.01129	0.03644
	HC (n=12)	7.3846	0.006578	0.01973
NEA (n=14)	HC (n=12)	9.3333	0.001451	0.008370

Table S2- Adonis/PERMANOVA analysis

	Df	Sums of Sqs	MeanSqs	F. Model	R2	<i>P-value</i>
QIIME Data	2	0.5137	0.25687	1.8201	0.09935	0.003**
Residuals	33	4.6571	0.14113		0.90065	
Total	35	5.1708			1	

Figure S4- Different distribution of the microbiota in taxonomic level of phylum.

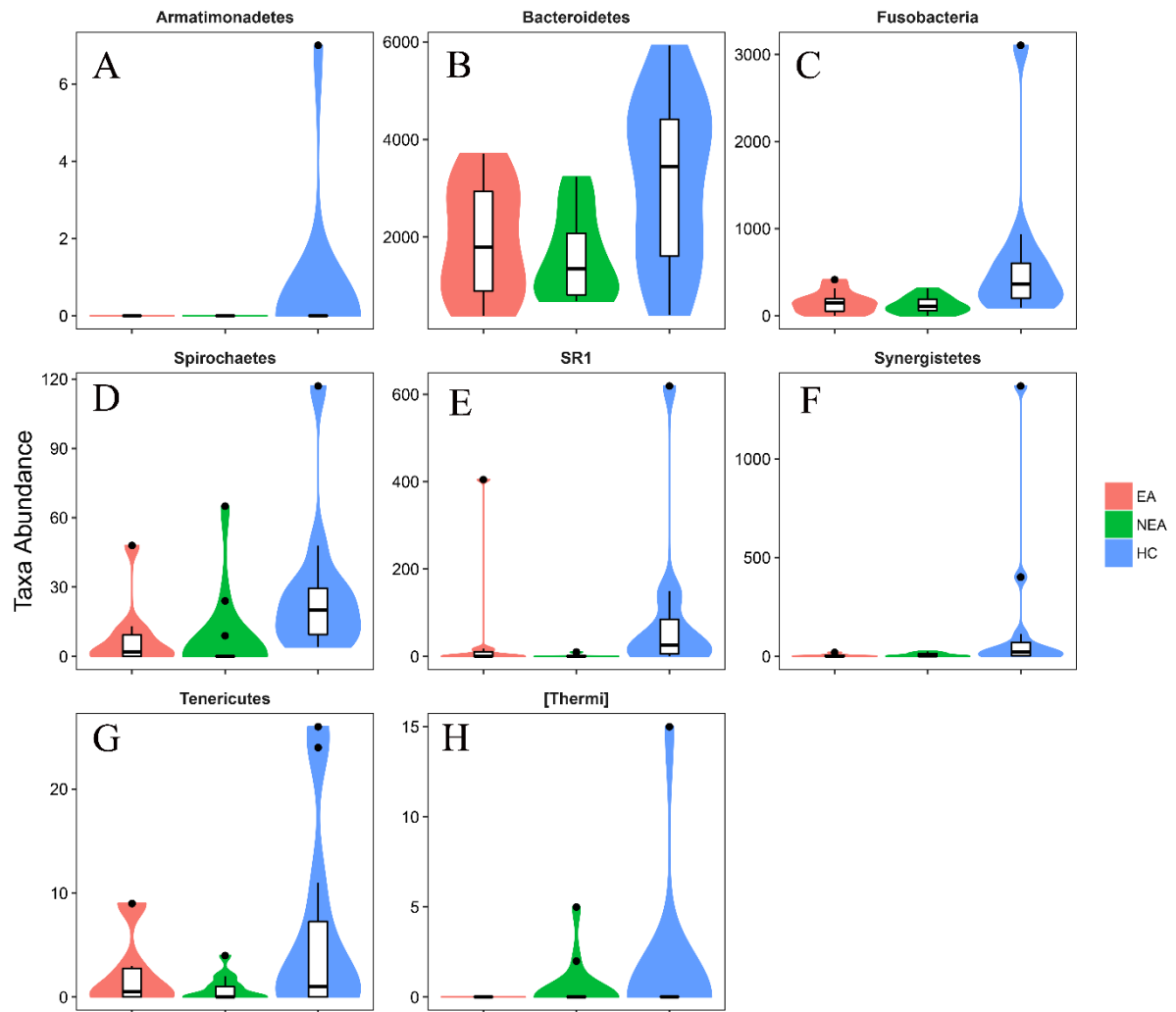


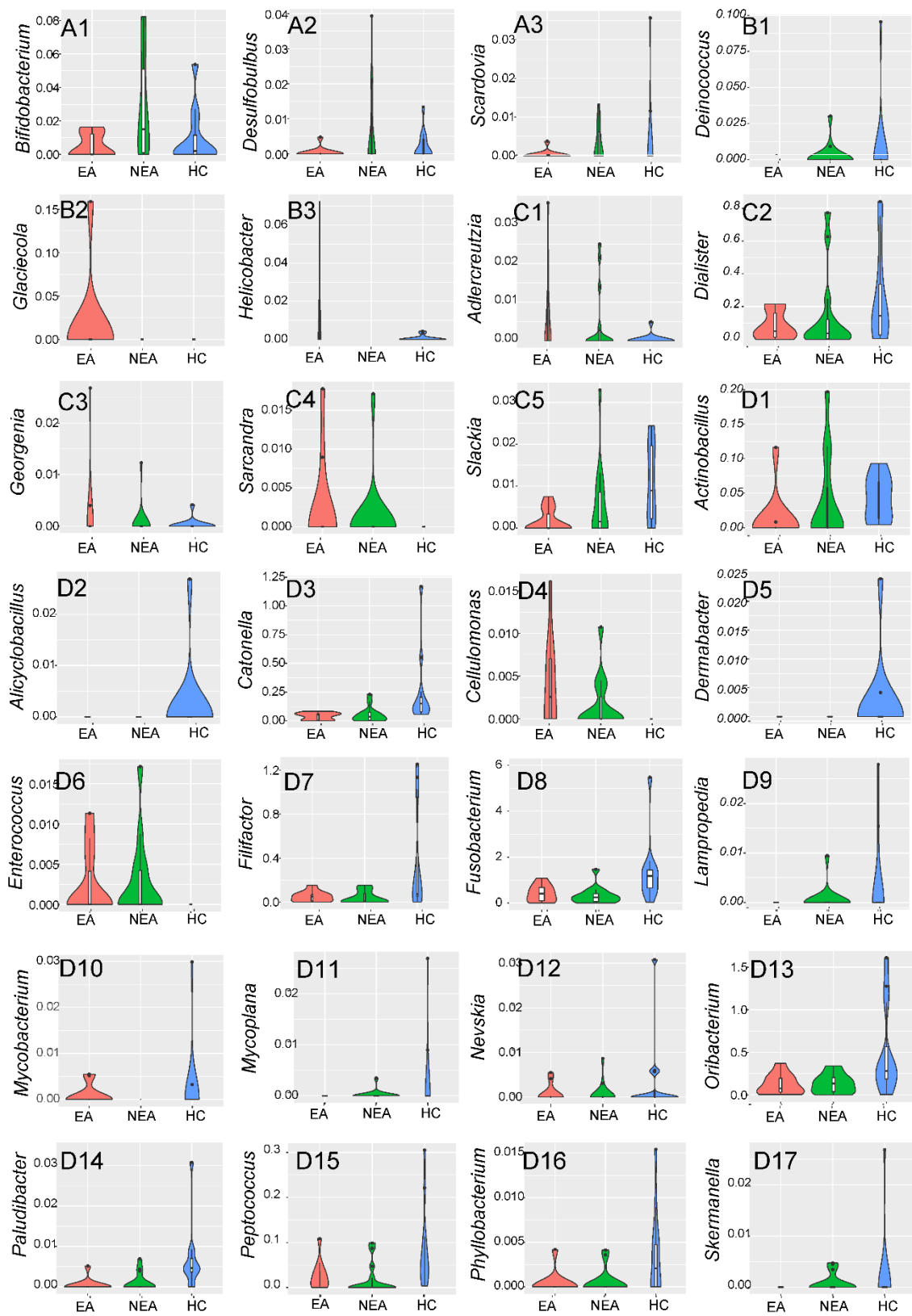
Figure S4 Different distribution of the microbiota in taxonomic level of phylum. Abundance of *Armatimonadetes* (**A**, EA vs HC $P=0.018$, NEA vs HC $P=0.004$), *Bacteroidetes* (**B**, EA vs HC $P=0.049$, NEA vs HC $P=0.008$), *Fusobacteria* (**C**, EA vs HC $P=0.007$, NEA vs HC $P=0.004$), *Spirochaetes* (**D**, EA vs HC $P=0.047$, NEA vs HC $P=0.032$), SR1 (**E**, NEA vs HC $P=0.001$), *Synergistetes* (**F**, EA vs HC $P=0.01$, NEA vs HC $P=0.021$), *Tenericutes* (**G**, NEA vs HC $P=0.034$), *Thermi* (**H**, NEA vs EA $P=0.046$) in three groups.

Table S3 Statistic Content level and changed fold of all phyla

Phyla	Levels	Changed Fold (EA/NEA/HC) §	P values†
<i>Armatimonadetes</i>	H>E≈N	0.073/0.073/1.0	<0.05
<i>Bacteroidetes</i>	H>E≈N	0.47/0.35/1.0	<0.05
<i>Fusobacteria</i>	H>E≈N	0.77/0.73/1.0	<0.05
<i>Spirochaetes</i>	H>N≈E	0.30/0.51/1.0	<0.05
<i>SRI</i>	H≈E<N	0.91/0.49/1.0	<0.001
<i>Synergistetes</i>	H>E≈N	0.58/0.63/1.0	<0.05
<i>Tenericutes</i>	E≈H>N	0.87/0.70/1.0	<0.05
<i>[Thermi]</i>	H≈N>E	0.067/0.89/1.0	<0.05

† *P* values were calculated statistically and followed by False Discovery Rate for comparison among the groups. § The changed fold values of distinct phyla were the ratios of the logarithmic transformation on the respective OTU ratios.

Figure S5- Different distribution of the microbiota in taxonomic level of genus.



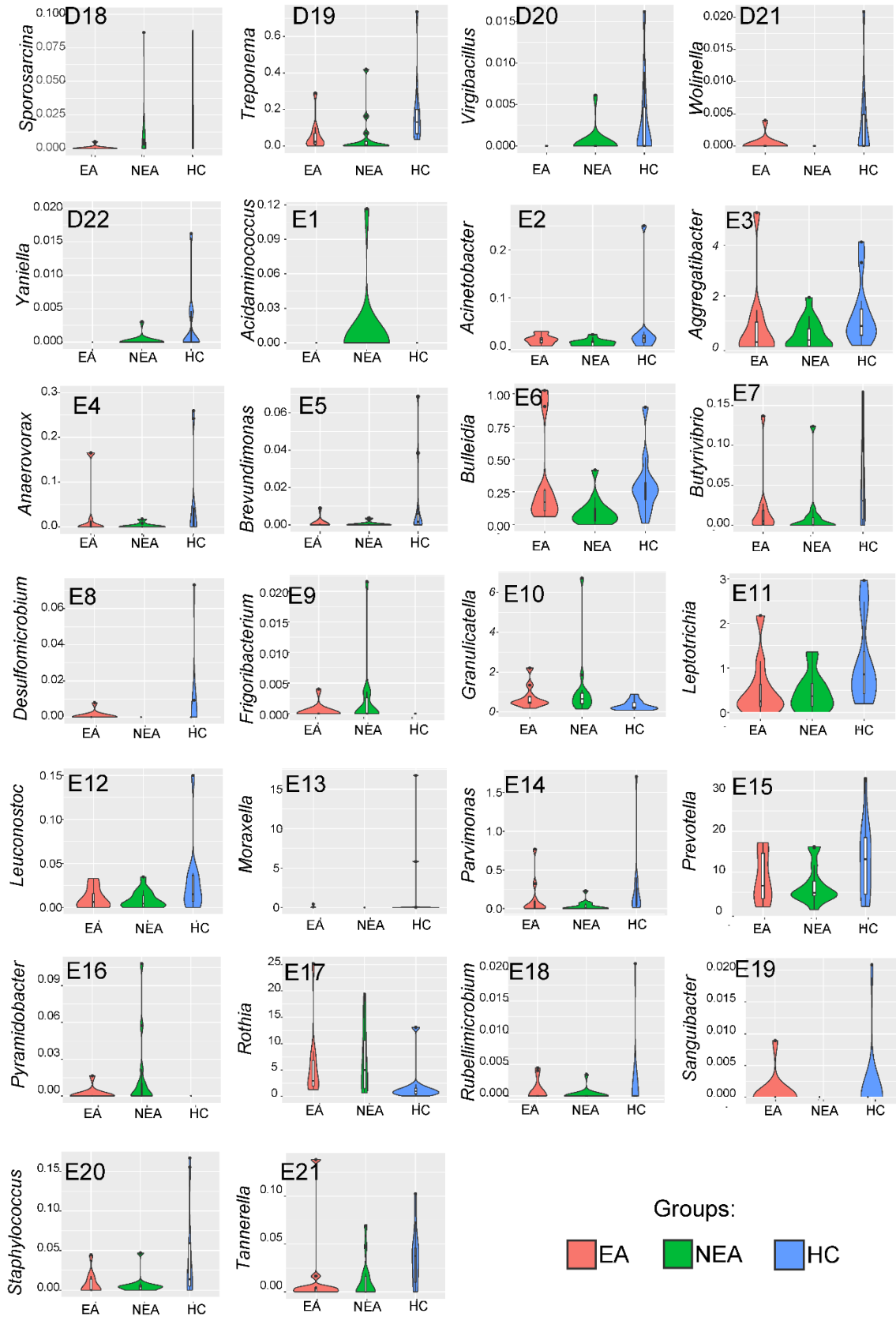


Figure S5 Different distribution of the microbiota in taxonomic level of genus. Abundance of *Bifidobacterium*, *Desulfobulbus* and *Scardovia* (A, EA vs NEA $P < 0.05$), *Deinococcus*,

Glaciecola and *Helicobacter* (**B**, EA vs NEA $P<0.05$ and EA vs HC $P<0.05$) , *Adlercreutzia*, *Dialister*, *Georgenia*, *Sarcandra* and *Slackia* (**C**, EA vs HC $P<0.05$) , *Actinobacillus*, *Alicyclobacillus*, *Catonella*, *Cellulomonas*, *Dermabacter*, *Enterococcus*, *Filifactor*, *Fusobacterium*, *Lampropedia*, *Mycobacterium*, *Mycoplana*, *Nevskia*, *Oribacterium*, *Paludibacter*, *Peptococcus*, *Phyllobacterium*, *Skermanella*, *Sporosarcina*, *Treponema*, *Virgibacillus*, *Wolinella* and *Yaniella* (**D**, EA vs HC $P<0.05$ and NEA vs HC $P<0.05$) , *Acidaminococcus*, *Acinetobacter*, *Aggregatibacter*, *Anaerovorax*, *Brevundimonas*, *Bulleidia*, *Butyrivibrio*, *Desulfomicrobium*, *Frigoribacterium*, *Granulicatella*, *Leptotrichia*, *Leuconostoc*, *Moraxella*, *Parvimonas*, *Prevotella*, *Pyramidobacter*, *Rothia*, *Rubellimicrobium*, *Sanguibacter*, *Staphylococcus* and *Tannerella* (**E**, NEA vs HC $P<0.05$) in three groups.

Table S4 Statistic Content level and changed fold of all genera

Groups	Genera	Levels	Changed Fold (EA/NEA/HC) [§]	P values [†]
A EA vs NEA	<i>Bifidobacterium</i>	N>E≈H	0.89/1.2/1.0	<0.05
	<i>Desulfobulbus</i>	N>E≈H	0.57/1.3/1.0	<0.001
	<i>Scardovia</i>	H≈N>E	0.44/0.96/1.0	<0.05
B EA vs NEA EA vs HC	<i>Deinococcus</i>	H≈N>E	0.067/0.89/1.0	<0.05
	<i>Glaciecola</i>	E>N≈H	16/1.0/1.0	<0.001
	<i>Helicobacter</i>	E>H>N	1.5/0.091/1.0	<0.01
C EA vs HC	<i>Adlercreutzia</i>	E>N≈H	1.4/1.3/1.0	<0.001
	<i>Dialister</i>	E<N≈H	0.69/0.85/1.0	<0.05
	<i>Georgenia</i>	E>N≈H	3.8/2.1/1.0	<0.05
	<i>Sarcandra</i>	E>N≈H	2.5/1.8/1.0	<0.05
	<i>Slackia</i>	E<N≈H	0.64/0.88/1.0	<0.01
D EA vs HC NEA vs HC	<i>Actinobacillus</i>	E≈N<H	0.85/0.83/1.0	<0.05
	<i>Alicyclobacillus</i>	E≈N<H	0.57/0.57/1.0	<0.05
	<i>Catonella</i>	E≈N<H	0.72/0.76/1.0	<0.05
	<i>Cellulomonas</i>	E≈N>H	14/12/1.0	<0.001
	<i>Dermabacter</i>	E≈N<H	0.076/0.076/1.0	<0.01
	<i>Enterococcus</i>	E≈N<H	0.12/0.11/1.0	<0.01
	<i>Filifactor</i>	E≈N<H	0.51/0.46/1.0	<0.05

	<i>Fusobacterium</i>	E≈N<H	0.68/0.64/1.0	<0.05
	<i>Lampropedia</i>	E≈N<H	0.073/0.21/1.0	<0.01
	<i>Mycobacterium</i>	E≈N<H	0.39/0.075/1.0	<0.05
	<i>Mycoplana</i>	E≈N<H	0.074/0.11/1.0	<0.001
	<i>Nevskia</i>	E≈N<H	0.68/0.64/1.0	<0.05
	<i>Oribacterium</i>	E≈N<H	0.85/0.85/1.0	<0.05
	<i>Paludibacter</i>	E≈N<H	0.39/0.53/1.0	<0.05
	<i>Peptococcus</i>	E≈N<H	0.85/0.79/1.0	<0.05
	<i>Phyllobacterium</i>	E≈N<H	0.47/0.54/1.0	<0.05
	<i>Skermanella</i>	E≈N<H	0.077/0.39/1.0	<0.01
	<i>Sporosarcina</i>	E≈N<H	0.31/0.69/1.0	<0.05
	<i>Treponema</i>	E≈N<H	0.65/0.61/1.0	<0.05
	<i>Virgibacillus</i>	E≈N<H	0.075/0.38/1.0	<0.001
	<i>Wolinella</i>	E≈N<H	0.35/0.073/1.0	<0.05
	<i>Yaniella</i>	E≈N<H	0.070/0.16/1.0	<0.05
E NEA vs HC	<i>Acidaminococcus</i>	N>E≈H	1.0/15.07/1.0	<0.0001
	<i>Acinetobacter</i>	N<E≈H	0.80/0.75/1.0	<0.05
	<i>Aggregatibacter</i>	N<E≈H	0.92/0.81/1.0	<0.05
	<i>Anaerovorax</i>	N<E≈H	0.91/0.71/1.0	<0.05
	<i>Brevundimonas</i>	N<E≈H	0.75/0.69/1.0	<0.05
	<i>Bulleidia</i>	N<E≈H	1.0/0.70/1.0	<0.05
	<i>Butyrivibrio</i>	N<E≈H	0.85/0.78/1.0	<0.05
	<i>Desulfomicrobium</i>	N<E≈H	0.77/0.068/1.0	<0.01
	<i>Frigoribacterium</i>	N>E≈H	1.21/1.43/1.0	<0.01
	<i>Granulicatella</i>	N>E≈H	1.22/1.33/1.0	<0.05
	<i>Leptotrichia</i>	N<E≈H	0.86/0.81/1.0	<0.05
	<i>Leuconostoc</i>	N<E≈H	0.89/0.80/1.0	<0.05
	<i>Moraxella</i>	N<E≈H	0.75/0.043/1.0	<0.001
	<i>Parvimonas</i>	N<E≈H	0.92/0.76/1.0	<0.05
	<i>Prevotella</i>	N<E≈H	0.85/0.76/1.0	<0.05
	<i>Pyramidobacter</i>	N>E≈H	1.2/3.4/1.0	<0.001
	<i>Rothia</i>	N>E≈H	2.3/2.5/1.0	<0.01
	<i>Rubellimicrobium</i>	N>E≈H	1.1/1.3/1.0	<0.05
	<i>Sanguibacter</i>	N<E≈H	0.92/0.079/1.0	<0.001
	<i>Staphylococcus</i>	N<E≈H	0.76/0.72/1.0	<0.05
<i>Tannerella</i>	N<E≈H	0.54/0.33/1.0	<0.05	

† *P* values were calculated statistically and followed by False Discovery Rate for comparison among the groups. § The changed fold values of distinct genera were the ratios of the logarithmic transformation on the respective OTU ratios.

Figure S6- Heatmap of the dominant 50 genera in all groups.

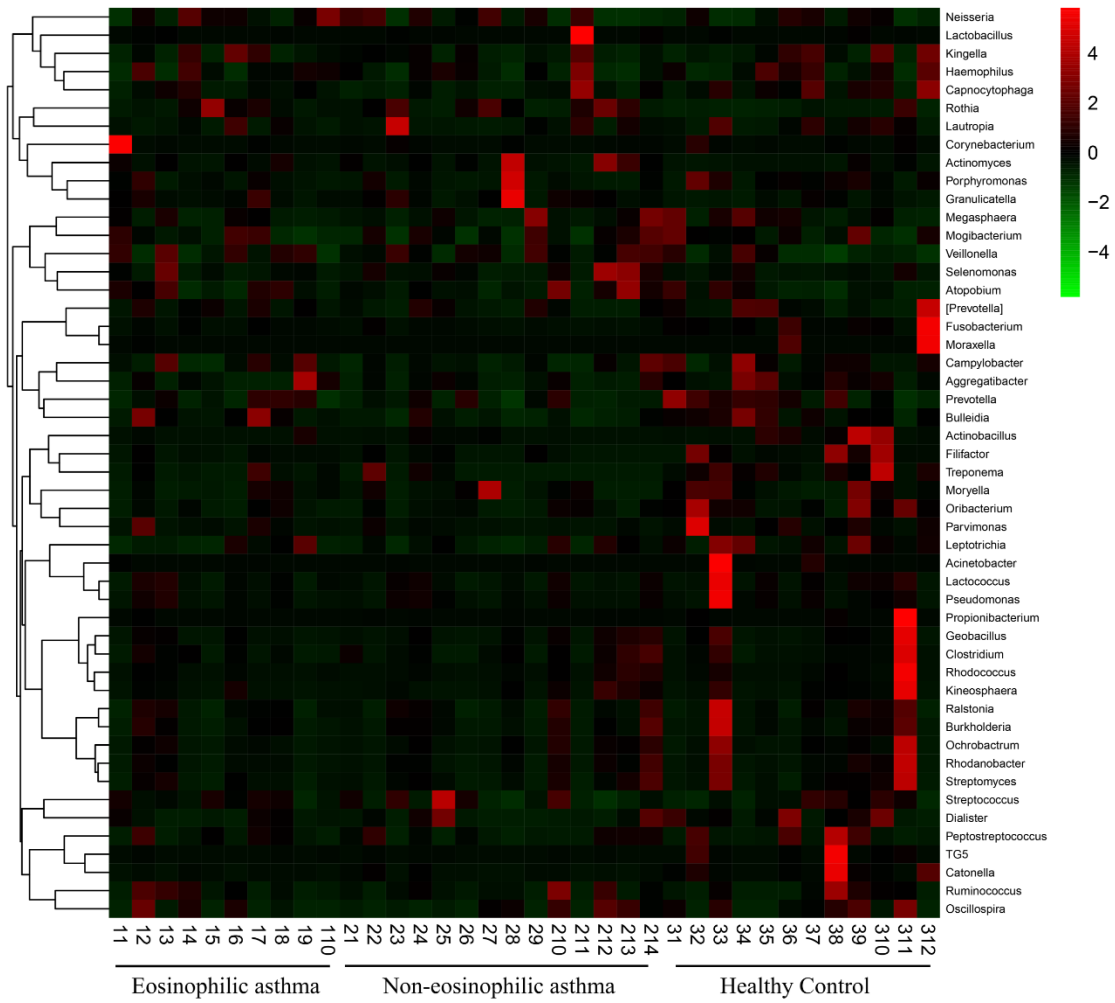


Figure S6 Heatmap of the dominant 50 genera in all groups.

Supplementary Information

Table S5- Prediction of microbial genes based on KEGG database

KEGG Pathway		EA	NEA	HC	P-value*	P_E vs. N	P_E vs. H	P_E vs. N
Cellular Processes	Cell Growth and Death	0.00538±0.000200	0.00556±0.000161	0.00568±0.000215	0.090	0.352	0.062	0.067
	Cell Motility	0.0109±0.00232	0.0113±0.00212	0.0137±0.00320	0.015	0.315	0.016	0.017
	Transport and Catabolism	0.00254±0.000170	0.00259±0.000432	0.00265±0.000305	0.120	0.353	0.159	0.352
Environmental Information Processing	Membrane Transport	0.114±0.0102	0.119±0.0127	0.111±0.0102	0.120	0.155	0.294	0.058
	Signalling Molecular and Interaction	0.00187±0.000238	0.00197±0.000342	0.00187±0.000175	0.221	0.211	0.473	0.182
	Signal Transduction	0.0125±0.000826	0.0128±0.00122	0.0133±0.00212	0.246	0.222	0.133	0.275
Genetic Information Processing	Folding, Sorting and Degradation	0.0274±0.00107	0.00266±0.00133	0.0270±0.00234	0.233	0.069	0.308	0.305
	Replication and Repair	0.0972±0.00356	0.0962±0.00421	0.0951±0.00812	0.157	0.259	0.213	0.346
	Transcription	0.0218±0.000846	0.0220±0.00129	0.0222±0.00128	0.105	0.368	0.236	0.357
	Translation	0.0660±0.00244	0.0653±0.00283	0.0637±0.00641	0.223	0.293	0.141	0.214
Human Diseases	Cancers	0.000954±0.0000498	0.000998±0.000230	0.000993±0.000145	0.100	0.251	0.195	0.479
	Cardiovascular Diseases	0.00000979±0.00000947	0.0000158±0.0000172	0.0000271±0.0000477	0.195	0.142	0.121	0.224
	Immune System Diseases	0.000821±0.000201	0.000841±0.000221	0.000723±0.000170	0.152	0.414	0.113	0.073
	Infectious Diseases	0.00363±0.000321	0.00357±0.000400	0.00376±0.000192	0.161	0.367	0.113	0.064
	Metabolic Diseases	0.00110±0.0000912	0.00110±0.0000838	0.00105±0.0000598	0.107	0.419	0.055	0.060
	Neurodegenerative Diseases	0.00310±0.00104	0.00287±0.00101	0.00279±0.000640	0.142	0.293	0.199	0.409
Metabolism	Amino Acid Metabolism	0.0986±0.00250	0.0979±0.00330	0.0980±0.00293	0.085	0.289	0.295	0.481
	Biosynthesis of other Secondary metabolites	0.00778±0.000396	0.00775±0.000319	0.00795±0.000492	0.234	0.434	0.205	0.127
	Carbohydrate Metabolism	0.0911±0.00406	0.0936±0.00683	0.0928±0.00585	0.212	0.153	0.214	0.382
	Energy Metabolism	0.05880±0.00249	0.0578±0.00254	0.0584±0.00268	0.172	0.185	0.374	0.284

	Enzyme Families	0.0200±0.000843	0.0200±0.000850	0.0202±0.000919	0.160	0.442	0.268	0.206
	Glycan Biosynthesis and Metabolism	0.0277±0.00238	0.0262±0.00328	0.0282±0.00459	0.183	0.124	0.376	0.111
	Lipid Metabolism	0.0273±0.00238	0.0276±0.00135	0.0289±0.00378	0.172	0.345	0.136	0.153
	Metabolism of Cofactors and Vitamins	0.0496±0.00288	0.0482±0.00316	0.0472±0.00372	0.124	0.135	0.056	0.240
	Metabolism of other Amino Acids	0.0168±0.000383	0.0169±0.000445	0.0168±0.00102	0.034	0.277	0.435	0.436
	Metabolism of Terpenoids and Polyketides	0.0172±0.00100	0.0173±0.00114	0.0182±0.00167	0.072	0.394	0.056	0.062
	Nucleotide Metabolism	0.0460±0.00222	0.0457±0.00180	0.0446±0.00366	0.236	0.494	0.196	0.164
	Xenobiotics Biodegradation and Metabolism	0.0171±0.00185	0.0179±0.00284	0.0204±0.00703	0.108	0.230	0.074	0.136
Organismal Systems	Circulatory System	0.000495±0.000326	0.000442±0.000301	0.000426±0.000192	0.083	0.342	0.272	0.439
	Digestive System	0.000571±0.0000974	0.000529±0.0000796	0.000655±0.000164	0.017	0.126	0.086	0.014
	Endocrine System	0.00283±0.000290	0.00312±0.00123	0.00301±0.000642	0.135	0.207	0.205	0.385
	Environmental System	0.00122±0.0000950	0.00121±0.000102	0.00126±0.0000596	0.164	0.415	0.113	0.066
	Excretory System	0.000245±0.000110	0.000279±0.000158	0.000213±0.000102	0.221	0.284	0.245	0.115
	Immune System	0.000299±0.000130	0.000316±0.000131	0.000516±0.000172	0.001	0.377	0.002	0.001
	Nervous System	0.000731±0.000119	0.000783±0.000207	0.000719±0.0000981	0.232	0.242	0.396	0.156
Others	Cellular Processes and Signalling	0.0420±0.00529	0.0404±0.00612	0.0409±0.00354	0.122	0.257	0.286	0.398
	Information Processing	0.0303±0.00154	0.0296±0.00205	0.0296±0.00229	0.184	0.177	0.206	0.492
	Metabolism	0.0221±0.000888	0.0220±0.000741	0.023±0.000881	0.002	0.386	0.008	0.001

* Multiple comparison with one-way analyses of variance (ANOVA). Data are shown as Mean ± SD. EA, NEA and HC are further abbreviated as E, N and H respectively.

All Supplementary Information items have been referenced by in-text citations in the main manuscript