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RESEARCH REPORT

Comparative characterization of bacterial communities in digestive glands of *Crassostrea gigas* fed with different microalgal diets

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Abstract

The digestive glands of marine molluscs are colonized by a large number of microorganisms, and the structure and function of bacterial community could be severely affected by diets. Microalgae is the main food and energy sources for bivalves, while the impact of phytoplankton composition on the bacterial community as well as the health of bivalves are still not well understood. In the present study, the bacterial communities in digestive glands of oyster *Crassostrea gigas* fed with different diets were compared based on the high-throughput sequencing of partial 16S rRNA gene. There were significant differences of bacterial composition rather than diversity in digestive glands between the oysters fed with diatom dominant diet (Group N, mainly made up of *Nitzschia closterium f.minutissima*) and dinoflagellate dominant diet (Group P, mainly made up of *Prorocentrum micans*). The abundances of *Prevotella*, *Vibronaceae*, *Ruminococcaceae*, and *Polaribacter* were significantly higher in Group N ($p < 0.05$), and the abundances of *Streptophyta* and *Acidimicrobiales* were significantly higher in Group P ($p < 0.05$). According to the functional prediction results, the bacterial community in Group P displayed weaker capacities of Kdo2-lipid A biosynthesis as well as taurine degradation, and a stronger capacity of glycolysis compared with the bacterial community in Group N. The higher phylogenetic clustering degree of the bacterial community in Group P ($p < 0.05$) indicated the higher host selectivity on bacteria. These results suggested that the change of phytoplankton composition of diet would have large effects on bacterial communities in oyster digestive glands. The bacterial community in digestive glands of oysters living in dinoflagellate dominant waters would produce harmful impact to hosts. The present study provided a new perspective to explore the potential mechanism for the massive mortalities of oysters.

Key Words: *Crassostrea gigas*, bacterial community, 16S rRNA, different diets

Introduction

The Pacific oyster (*Crassostrea gigas*) has been cultivated globally as a key commercial aquaculture species since the 1950s. According to the latest statistical data, *C. gigas* accounted for more than 35 % production in China marine bivalve aquaculture industry (China Fishery Statistical Yearbook, 2019). In the last decade, large-scale mortalities occurred repeatedly in oyster aquaculture throughout the world (Li *et al*., 2007; Malham *et al*., 2009; Wendling and Alfred, 2013; Lorgeril *et al*., 2018). Current researches about the dominant reasons of this phenomenon mainly focused on OsHV-1-related disease, lethal heat shock in summer, reproduction depleting energy, and the increase of *Vibrio* abundance (Berthelin *et al*., 2000; Davison *et al*., 2005;

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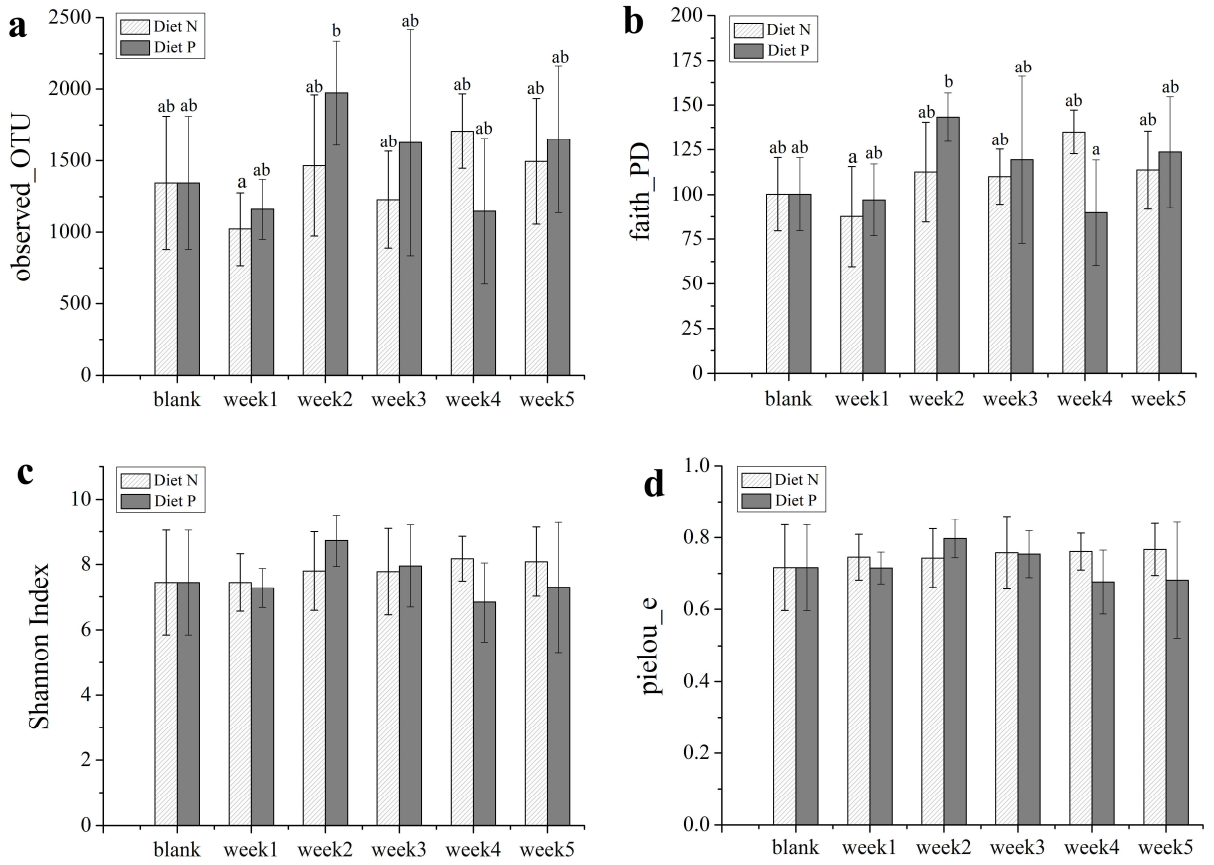

Fig. 1 The α-diversity indices of bacterial communities from Diet N and Diet P in five time points during the experiment. a observed_OTU index of different samples. b pielou_e index of different samples. c faith_PD index of different samples. d Shannon index of different samples. Lowercase letters above the error bars indicated statistical significance among samples

Friedman *et al*., 2005; Malham *et al*., 2009; Lorgeril *et al*., 2018; Timothy *et al*., 2018). The opinion that emphasized the ponderance of intense fluctuation of chlorophyll content and phytoplankton particles quality was the most common and compelling (Berg and Newell, 1986; Su *et al*., 2018). It has been suggested that the change of food composition can alter the mRNA expression and activity of digestive enzymes in oyster digestive gland, which can further affect the digestion and absorption capability of oysters (Moal *et al*., 2000; Huveta *et al*., 2003). However, the knowledge about the effect of diets on microbiome in digestive glands of *C. giga*s is limited, and the impact of microbiome on the host is still not well understood. It is necessary to study the variation in bacterial community structure and function in digestive glands of oysters fed with different diet supply, which is helpful to find the clue of large scale mortality and to improve the development of oyster farming industry.

Microbiome (host associated microbial community) is beneficial for animals in various aspects, such as regulating the host's immune response, development and physiology processes in mammals, and modulating locomotor behavior and environmental resilience in invertebrates (Hooper and Gordon, 2001; MacDonald and Monteleone, 2005; Wang *et al*., 2007; Erwin *et al*., 2012; Sommer and Bäckhed, 2013; Schretter *et al*., 2018). The abundant and diverse microbiome in invertebrates is beneficial for the host in terms of digestion and storage of nutrient, adaptation for stressful environmental conditions and defense against pathogen invasion (Zimmer *et al*., 2001; Olson and Kellogg, 2010; Sweet and Bulling, 2017). In sponge, microbiome was considered to play a crucial role in mediating the capacity of the host acclimation to environment changes (Pita *et al*., 2018). It has been reported that the abundant microbial communities inhabiting circulatory system can coexist with immunocompetent cells and prevent the establishment of potential pathogens by offering a competitive environment in oysters (Paulina *et al*., 2012). The digestive gland (hepatopancreas) is an integrated organ with immune and digestive functions for bivalves, which is also colonized by abundant microorganisms (Rőszer, 2014; Rubiolo *et al*., 2018).

As filter-feeding animals, bivalves obtain energy by filtering organic detritus in water, and their survival and growth are strongly influenced by phytoplankton community (Frankic and Hershner,

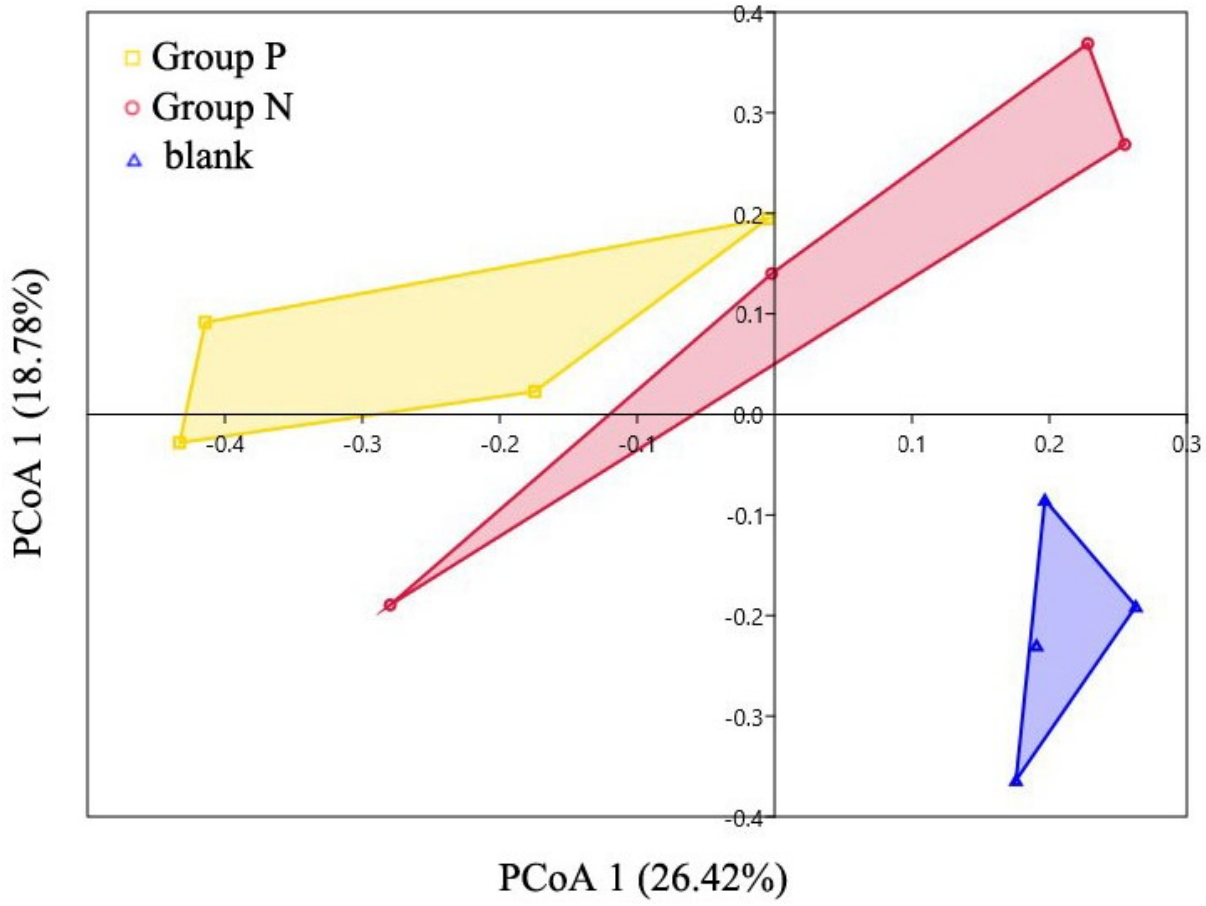

Fig. 2 The PCoA plot compared the bacterial community structures based on weighted Bray-Curtis distance metric

2003). The North Yellow Sea is one of the most important bivalves farming areas in China, where the phytoplankton community is mainly composed of diatoms and dinoflagellates (Liu *et al*., 2013). The dominant phytoplankton group has been reported to change from diatom to dinoflagellate in the Yesso scallop (*Patinopecten yessoensis*) farming waters in the North Yellow Sea during the recent years (Yu *et al*., 2019). Diatoms are important food and energy sources for bivalves, while dinoflagellates are often regarded as one of the main microalgae groups in harmful algae blooms, and some dinoflagellate species can even cause pathological damages to gut, muscle and gill of oysters and scallops (Wikfors and Smolowitz, 1995; Frankic and Hershner, 2003; Smayda and Reynolds, 2003; Imojen *et al*., 2005; Silina and Zhukova, 2007; Hégaret *et al*., 2012). The alternation of phytoplankton communities results in the change of food composition and living environment, which are suspected to contribute to the mortalities of *P. yessoensis* (Yu *et al*., 2019). In the present study, *C. gigas* was fed with diatom dominant diet and dinoflagellate dominant diet, respectively. The digestive gland was collected, and the compositions of the bacterial communities were investigated by high-throughput sequencing of V4 region of 16S rRNA gene with the objectives to (1) gain a comprehensive understanding of microbiome in digestive glands of *C. gigas*, (2) reveal the differences of microbiome assemblages in digestive glands of oysters fed with different diets, and (3) explore the effects of the changed bacterial community on oysters.

Materials and methods

Experiment design and sample collection

Prorocentrum micans GY-H39 and *Nitzschia closterium f. minutissima* were obtained from Institute of oceanology, Chinese academy of sciences and Ocean University of China, respectively. The microalgae cells were inoculated in 100 L of f/2 medium and cultured at 24 °C with a light-dark cycle of 12 h:12 h until the concentration reached 5 × 10^7 cells/mL (Lananan *et al*., 2013). Two kinds of microalgae mixtures were formulated as diets for oysters. The diatom dominant diet (designated as Diet N) was composed of 80 % *N. closterium* and 20 % *P. micans*, and the dinoflagellate dominant diet (designated as Diet P) was composed of 80 % *P. micans* and 20% *N. closterium.* The oysters were fed with each diet at the final concentration range from 5 × 10^5 to 7 × 10^5 cells/mL.

A total of 93 adult oysters were acquired from a local market in Dalian, Liaoning Province, China. They were maintained in aerated seawater for a week without feeding before experiments. After acclimatization, the oysters were divided into two groups, which were fed with Diet N and Diet P respectively once a day for five weeks. Every week, the digestive glands from three oysters were sampled using scalpels and tweezers under sterile condition and pooled together as one sample. There were three replicates for each time point. Samples collected at the last week of the experiment were designed as Group N (fed with Diet N) and Group P (fed with Diet P), respectively.

Genomic DNA extraction and high-throughput sequencing

The total genomic DNA was extracted from all samples using the E.Z.N.A. soil DNA Kit (Omega, Norcross, GA, USA) according to manufacturer's recommended protocol. Concentration and purity of DNA were examined by NanoDrop spectrophotometer (Thermo Fisher Scientific, Inc., Wilmington, DE, USA) (Supplementary materials Table S1). The V4 hypervariable region of 16S rRNA gene was amplified using the primers of 515F (GTGBCAGCMGCCGCGGTAA) and 806R (GGACTACHVGGGTWTCTAAT) (Kumar *et al.*, 2015). The raw sequence reads generated by high-throughput sequencing (HTS) on Ion S5 XL platform (Novogene, Beijing, China) were demultiplexed and converted to FASTQ format.

Diversity analysis and function prediction of the bacterial communities

The high-throughput sequencing data were imported into the QIIME2 platform (v2018.11) and processed by the Deblur program (Amir *et al*., 2017; Bolyen *et al*., 2019). After denoised by Deblur, sequences are usually called amplicon sequence variants (ASVs), exact sequence variants (ESVs), or sub-operational taxonomic units (sub-OTUs). In the present study, these sequences were assigned to bacterial features, which were synonymous to ASVs, ESVs and sub-OTUs, and sequences between different features differed at the single-nucleotide level. The taxonomy of these features was assigned to the Greengenes reference database (13-8 version) classifier with 99% similarity (Desantis *et al*., 2006).

The α-diversity indices including observed OTUs, faith_PD, Shannon and pielou_e index, and the composition of the bacterial communities as well as the rarefaction curves were calculated and visualized. For β-diversity, the rarefied feature table was used to determine the Bray-Curtis distance matrix values for performing non-parametric statistical analysis by ANOSIM (analysis of similarities) with 999 permutations using the Vegan (v.2.5.5) R package (Bray and Curtis, 1957; Dixon, 2003). Principle Coordinates Analysis (PCoA) using Bary-Curtis distance metric was performed to visualize separation of samples. Phylogenetic investigation of Communities by Reconstruction of Unobserved States (PICRUSt2; https://github.com/picrust/picrust2) was used to predict the functional potential of prokaryotic communities based on the feature table and the representative sequence data from Greengenes reference database (13-8 version). The prediction of KO abundances was carried out with Hidden-state prediction, and the KOs were collapsed into pathways (Zaneveld and Thurber, 2014). The statistical analysis of metagenomic profiles (STAMP) (version 2.1.3) package was employed for further statistical analysis and visualization.

Community phylogenetic analysis

To explore the ecological process of host linked bacterial community colonization, mean nearest taxon distance (MNTD) was calculated (Chu *et al*., 2016). The standardized effect size of MNTD (ses.MNTD) was used to quantify the number of standard deviations of the observed MNTD from the mean of the null distribution (999 randomizations) (Wang *et al*., 2013). Negative ses.MNTD values ($p < 0.05$) indicates that co-occurring bacterial species are more closely related than expected by chance (phylogenetic clustering) (Wang *et al*., 2013). Abundance-weighted ses.MNTD was calculated by the package Picante 2.5-5 using a null model which held richness constant (Richness Randomization) in the R environment (http://www.r-project.org) (Kembel *et al*., 2010).

Statistical analysis

Linear discriminant analysis effect size (LEfSe) method was used to identify significantly different abundant bacterial genus between Group N and Group P, which was performed online in the Galaxy workflow framework (https://huttenhower.sph.harvard.edu/galaxy/) (Segata *et al*., 2011). The alpha value for the factorial Kruskal-Wallis test was set to 0.05, and the threshold on the logarithmic LDA score for discriminative features was set to 3.0. Statistical Package for Social Sciences (SPSS) 19.0 (SPSS INC, Chicago, IL, USA) was used for the statistical analysis. Significant differences among multiple groups were tested with One-way analysis of variance (ANOVA), followed by Duncan's post hoc test. Significant differences between two groups were tested with student's t-test. One asterisk (*) indicated statistical significance ($p < 0.05$), and double asterisks (**) indicated extremely statistical significance ($p < 0.01$).

Results

α- and β-diversity of bacterial communities

High-throughput sequencing of 16S rRNA gene V4 region was performed to characterize the bacterial lineages in digestive glands of oysters fed with Diet N and Diet P. There were 1,432,748 reads in total, which were clustered into 14,267 features (Supplementary materials Table S2). The rarefaction curves tended to approach the saturation plateau, suggesting that sequencing depth was sufficient for all samples (Supplementary materials Fig. S1).

The number of species and phylogenetic diversity (indicated by observed_OTU index and faith_PD index, respectively) (Fig. 1a, b) were compared between the Diet N feeding group and Diet P feeding group. The number of species was higher in Diet P feeding group after two weeks

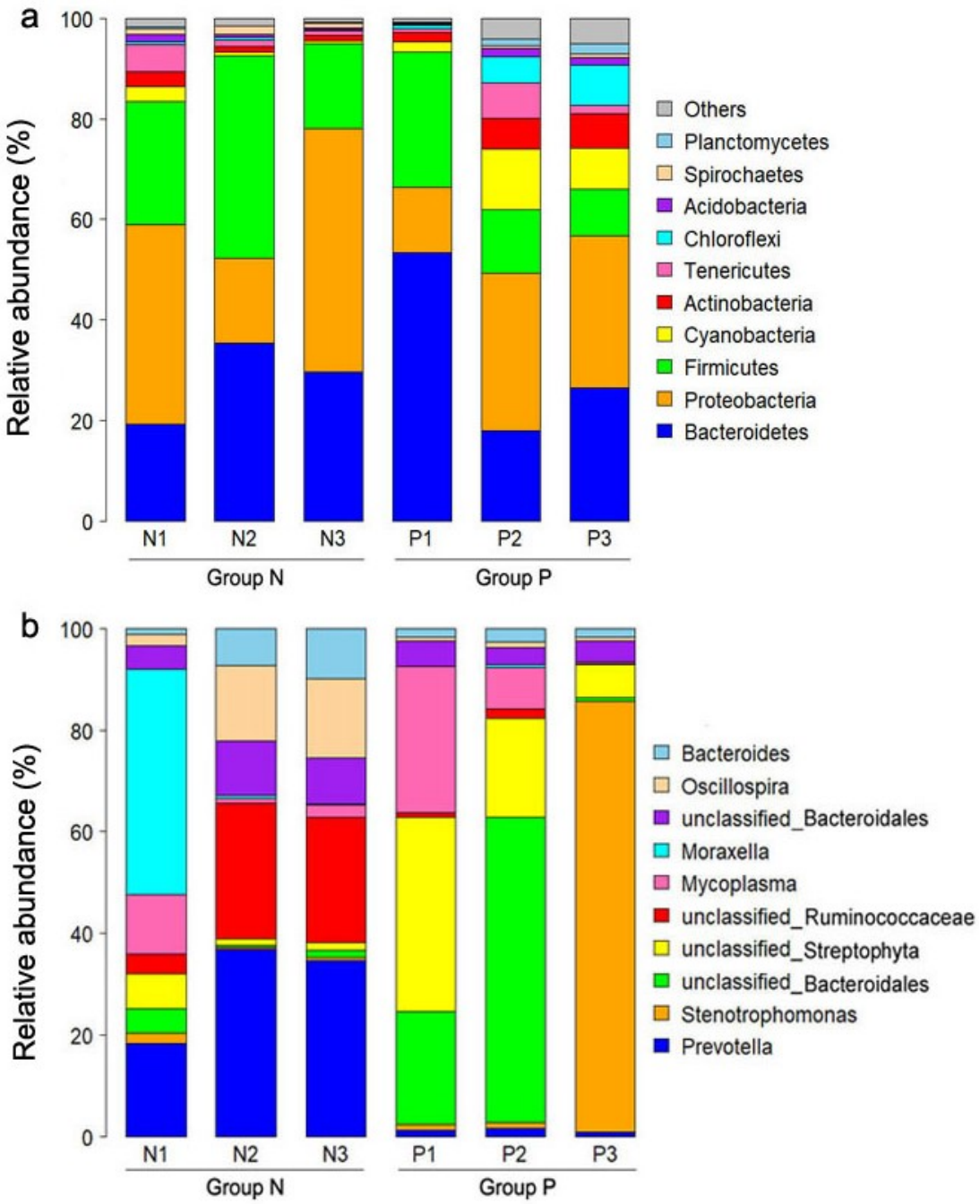

Fig. 3 Bacterial community compositions of Group N and Group P. a Bacterial community compositions at phyla level, the top 10 abundant groups were shown in the figure and the rest was indicated as “Others”. b Bacterial community compositions at genus level, the top 10 abundant groups were shown in the figure

feeding and lower in Diet N feeding group after one week feeding. The phylogenetic diversity was higher in Diet P feeding group after two weeks feeding and lower in Diet N and Diet P feeding group after one week 0 and four weeks feeding, respectively. There were no significant differences of community diversity and community evenness (indicated by Shannon index and pielou_e index, respectively) between the two groups during the experiment (Fig. 1c, d).

As there was no significant change in the structure composition and diversity between the two groups in the first four weeks, the data of the Group N and Group P at the fifth week were further analyzed. PCoA plot showed that the bacterial community structures were clearly separated from each other (Fig. 2) after different diets feeding for five weeks, which was confirmed by the ANOSIM analysis ($R = 0.4421$, $p = 0.0053$).

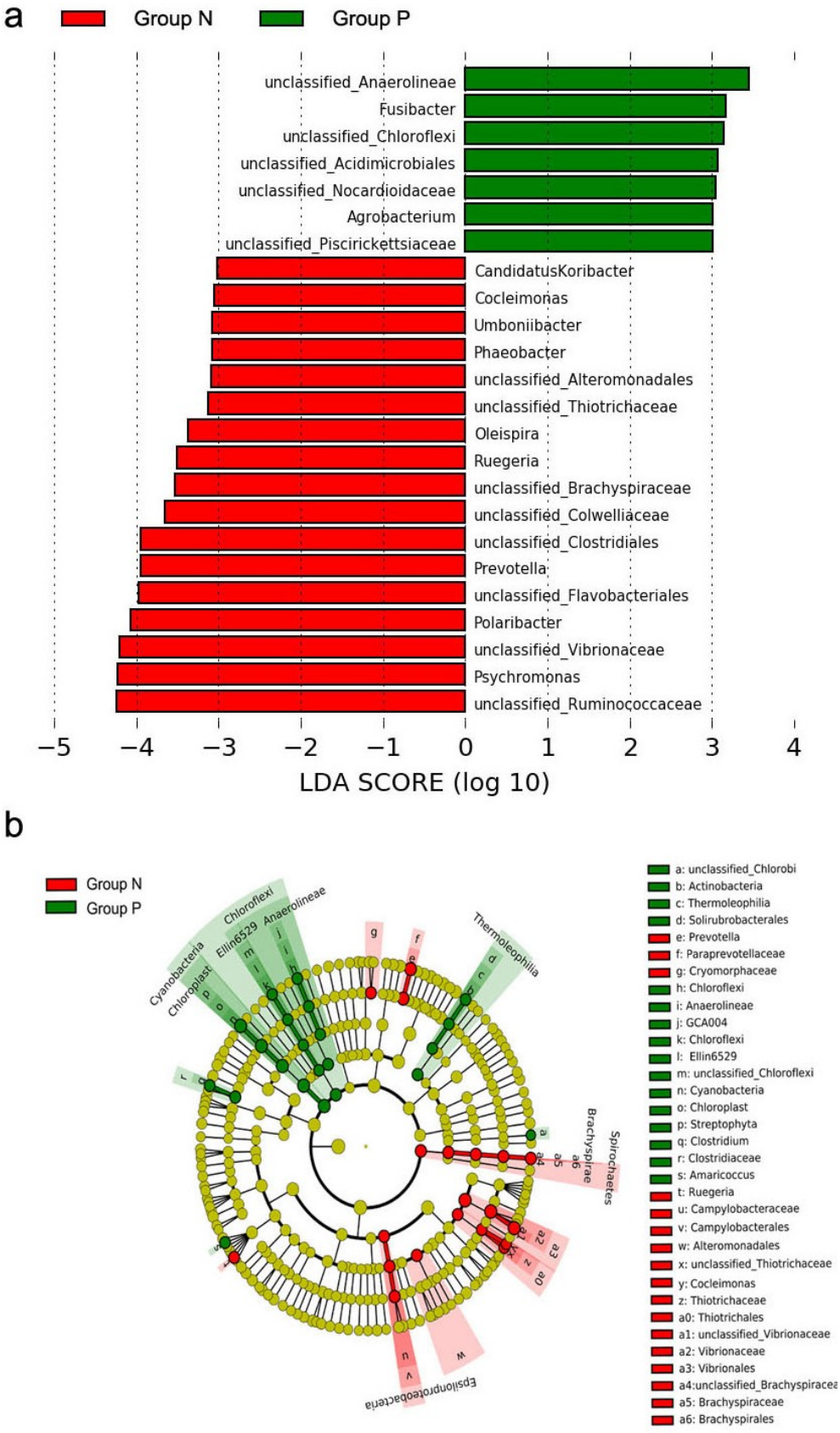

Fig. 4 LEfSe statistics in Group N and Group P. a Chart plotted from LEfSe analysis showed the bacterial taxa have significantly relative abundance difference. b Cladogram plotted from LEfSe analysis showed the taxonomic levels represented by rings with phyla in the outermost the ring and genera in the innermost ring. Each circle was a member within that level. Those taxa in each level were colored by group for which it was more abundant ($p < 0.05$, LDA score = 3)

The composition differences of bacterial communities

Composition of bacterial communities in digestive glands in Group N and Group P were analyzed according to the annotated feature table. Roughly, 99 % of total reads were annotated at phylum level, and 56 % of total reads were annotated at genus level. There were ten dominant phyla which accounting for about 80 % relative abundance in total across all samples, and they had different proportions in two groups (Fig. 3a). *Bacteroidetes*, *Proteobacteri*a, and *Firmicutes* were the most dominant phyla in digestive glands of both Group N and Group P bacterial communities. The abundances of *Cyanobacteria*, *Actinobacteria,* and *Chloroflexi* were higher in digestive glands of Group P, and the abundance of *Acidobacteria* was higher in digestive glands of Group N.

The relative abundances of the top ten genera in digestive glands of Group N and Group P were analyzed (Fig. 3b). The abundances of *Prevotella*, *Oscillospira, Bacteroides*, unclassified *Ruminococcaceae*, and unclassified *Bacteroidales* were higher in Group N, while the abundances of *Mycoplasma*, unclassified *Bacteroidales*, and unclassified *Streptophyta* were higher in Group P.

LEfSe analysis identified 24 phylotypes whose relative abundances were significantly affected by different diets of microalgae (Fig. 4). The relative abundance of 17 genera in Group N were enriched (p < 0.05), and eight of them accounted for more than 1 % relative abundance, which were *Prevotella* within the family *Prevotellaceae*, *Polaribacter* within the family *Flavobacteriaceae*, *Psychromonas* within the family *Psychromonadaceae*, unclassified *Colwelliaceae*, unclassified *Vibrionaceae*, unclassified *Ruminococcaceae*, unclassified *Flavobacteriales*, and unclassified *Clostridiales*. The relative abundances of seven genera were significantly higher (p < 0.05) in Group P (relative abundance < 1 %), which were *Fusibacter* within the family *Acidaminobacteraceae*, *Agrobacterium* within the family *Rhizobiaceae*, unclassified *Anaerolineae*, unclassified *Chloroflexi*, unclassified *Acidimicrobiales*, unclassified *Nocardioidaceae*, and unclassified *Piscirickettsiaceae*.

The functional distinctions of bacterial communities

The functions of bacterial community in the digestive glands of oysters fed with different diets were predicted by PICRUSt program based on KEGG pathways. The nearest sequenced taxon index (NSTI) value of all samples in the PICRUSt analysis ranged from 0.054 to 0.265 with a mean value of 0.196 (Supplementary materials Table S3). According to statistical data, there were 13 KEGG pathways exhibiting significantly different abundance between the two groups (p < 0.05) (Fig. 5). Among them, seven pathways were enriched in Group N, which associated with aspartate pathway, coenzyme M biosynthesis, superpathway of sulfolactate degradation, 4-hydroxyacetophenone degradation, superpathway of (Kdo)2-lipid A biosynthesis, peptidoglycan biosynthesis II, and superpathway of taurine degradation. Six pathways were enriched in Group P, which were related to queuosine biosynthesis, GDP-mannose biosynthesis, glycolysis II, fatty acid elongation-saturated, phosphopantothenate biosynthesis I, and vitamin B6 degradation.

Phylogenetic distinctions of bacterial communities

MNTD values and the ses.MNTD values were calculated to analyze the phylogenetic relationship of the bacterial community composition (Fig. 6). The ses.MNTD values were significantly negative (p = 0.001, 999 permutations), indicating phylogenetically clustering of bacterial communities in both Group N and Group P. The absolute values of ses.MNTD in Group P (5.469 in average) was significantly higher than that in Group N (3.119 in average) (p < 0.05), which suggested a stronger phylogenetic clustering degree of the bacterial community after feeding with Diet P for five weeks.

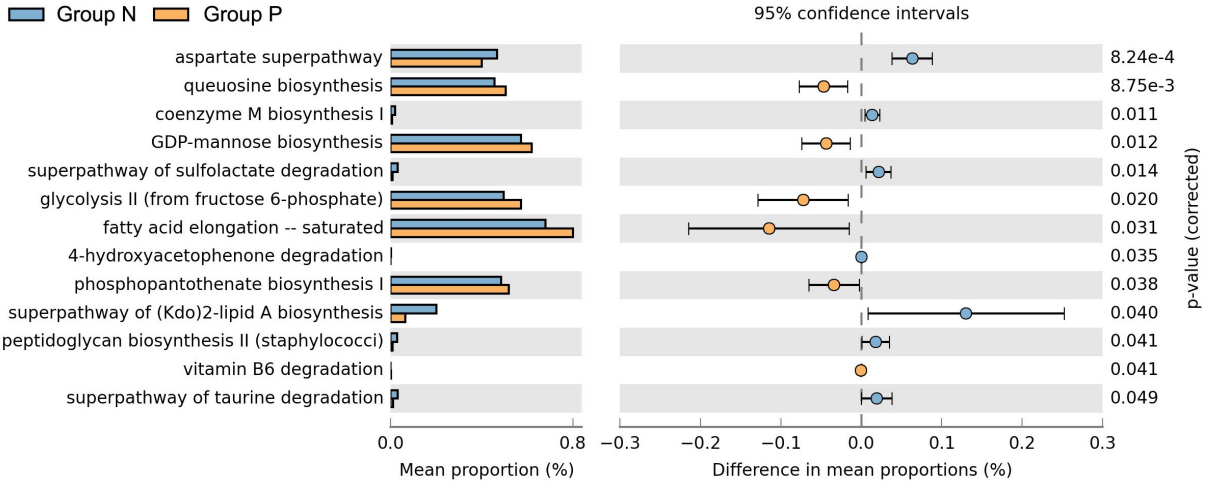

Fig. 5 Predicted function of bacterial community. STAMP analysis based on the PICRUSt dataset revealed differentially enriched metabolic pathways associated with Group N and Group P

Discussion

The abundance and composition of phytoplankton in North Yellow sea has dramatically changed from diatom dominant type to dinoflagellate dominant type in recent years, which is considered to be one of the main reasons affecting oyster production (Liu *et al*., 2013). The chemical compositions of diatom and dinoflagellate are quite different, and the specific impact of the phytoplankton community alteration on oyster health is still not clear (Kwok and Wong, 2015; Valentin and Inna, 2015). In the present study, the influence of different diets on the bacterial community in oyster digestive glands was investigated, which was helpful to find the connections between diets and symbiotic bacterial community, and provided a new perspective for further unveiling the mechanism of oyster massive mortalities.

In the present study, different composition of microalgae diets led to significant distinctions of structure and composition rather than diversity of bacterial communities in oyster digestive glands, which was suspected to due to great differences of chemical composition and digestion degree between *P. micans* and *N. closterium*. The present results were similar to the previous reports in *Ochotona curzoniae* and *Felis catus* that different contents of diets were more likely to cause differences of β-diversity than α-diversity of gut microbiota and faecal bacteriome (Huan *et al*., 2016; Butowski *et al*., 2019). For the composition of bacterial communities, there were significant rises in the relative abundances of *Prevotella*, *Polaribacter*, and *Ruminococcaceae* in diatom dominant diet group compared with dinoflagellate dominant diet group. *Prevotella* was reported to be strongly associated with carbohydrates in diets and played a direct role in improving glucose metabolism and promoting glycogen storage potentially (Petia *et al*., 2015). *Polaribacter* was also ascribed to act as degraders of biopolymers such as polysaccharides since it featured high proportions of glycoside hydrolase genes screened from genomic data, and these results were falling in line with high *Polaribacter* abundances in diatom-rich habitats (Thomas *et al*., 2011; Teeling *et al*., 2012; Mann *et al*., 2013). *Ruminococcaceae* was well illustrated to be responsible for the degradation of diverse polysaccharides and production of short chain fatty acid (SCFA) as beneficial symbiotic bacteria (Shang *et al*., 2016). Therefore, the high abundances of *Prevotella*, *Polaribacter*, and *Ruminococcaceae* in digestive glands of oysters fed with diatom dominant diet group provided strong capabilities to degrade and metabolize polysaccharides of diatom. Compared with diatom dominant feeding group, there was no enrichment of biopolymer degrading bacteria in dinoflagellate dominant feeding group even though dinoflagellates accumulated abundant starch and glycogen as main storage carbonhydrates (Deschamps *et al*., 2008). The cell wall and protoplast of *N. closterium* are full of polysaccharides, and the content of polysaccharides is higher than that of proteins or lipids in the cell wall

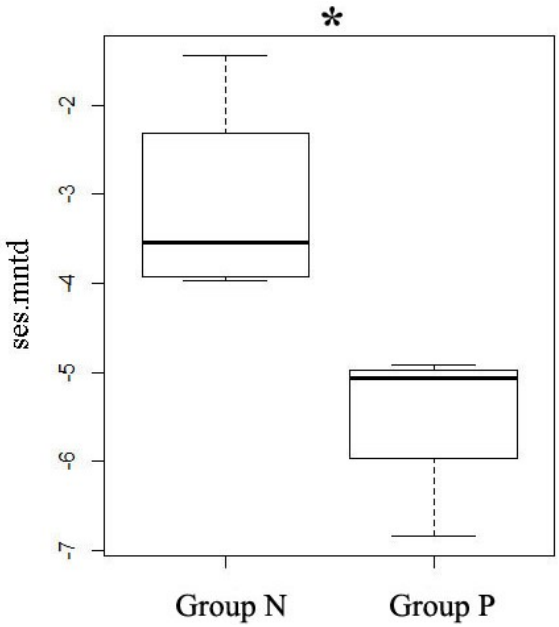

Fig. 6 Variation of standardized effect sizes of mean nearest taxon distance (ses.MNTD) of bacterial communities in Group N and Group P

(Bruno *et al*., 2015). Compared with *N. closterium*, thick cellulose thecae or cell covering structure enveloping the whole cell made *P. micans* almost no effective nutrition component for oysters (Rey *et al*., 2001). It has been observed that the oysters farmed in the sea area dominated by *P. micans* basically couldn't gain weight in several months (King *et al*., 2006). Indigestive thecae of *P. micans* made starch and glycogen could not become the main nutrition source for oysters, and some important functional bacteria also could not be enriched in oyster digestive glands feeding with dinoflagellate dominant diet. Producing metabolites is the main pattern of bacteria to interact with the host (Chang *et al*., 2013). The alternation of bacterial communities and the variation or deficiency of bacterial metabolites would inevitably lead to the downturn of metabolism, immunity, and physiology conditions.

It has been demonstrated that the change of microbiome phenotypes could lead to different potential function phenotypes of the host. PICRUSt has been used to accurately predict the function of human and environmental microbiomes. The accuracy of the prediction decreases with increasing NSTI value, and reliable results were generated from a dataset of soil samples with a mean NSTI score of 0.17 (Wang *et al*., 2016). In the present study, the mean NSTI value was 0.196, indicating that there was much unexplored bacterial diversity in the digestive gland of *C. gigas*. Function prediction indicated that the abundances of genes involved in Kdo2-lipid A biosynthesis as well as taurine degradation were significantly lower, while the abundance of genes involved in glycolysis pathway was significantly higher in dinoflagellate dominant

feeding group than that in diatom dominant feeding group. Kdo2-lipid A is an essential structural component of lipopolysaccharide (LPS) and serves as a powerful innate immune system activator through binding to the heterodimer of Toll-like-receptor 4 (TLR4) and myeloid differentiation protein 2 (MD-2) (Park *et a*l., 2009; Wang *et al*., 2014). Taurine has been reported to participate in regulation of intracellular osmolality and cell volume as a kind of free amino acids (FAAs), which contribute effectively to oyster euryhaline adaptation (Pierce and Amende, 1981). The metabolic pathway expansion and up/downregulation of FAAs were proved to be more important effectors of oyster euryhaline adaptation (Meng *et al*., 2013). Compared with *N. closterium*, *P. micans* could hardly provide nutrition for the thick indigestible cellulose thecae. Under the poor-nutrition condition of dinoflagellate dominant feeding group, the hepatic glycogen and muscle glycogen were invoked urgently to transform to glucose and to meet the energy needs of oyster normal physiological processes. However, the significantly high abundance of glycolysis pathway related genes of bacterial community provided the evidence that bacteria competed glucose with their host. In general, the bacterial community in dinoflagellate dominant feeding group could fatigue host immune system and thus decrease its sensitivity to the invasion of bacteria pathogens, weaken host euryhaline adaptation capability and compete glucose energy with their host.

From a phylogenetic perspective, the bacterial communities from Group N and Group P were phylogenetically clustered. The symbiotic bacteria lineages in insects and sponges can converge functionally to offer or maintain functions which contribute to host survival or to stabilize host-microbiome interaction as the symbiotic relationship evolves (Moran and Yun, 2015; Pita *et al*., 2018). Meanwhile, hosts could acquire biosynthetic and degradation functions from distinct bacterial communities (Hongoh, 2011). Environmental disturbance and virus invasion seriously influence symbiotic bacterial community assembly and phylogenetically clustering, which are closely associated with bivalve health (Wegner *et al*., 2013; Lorgeril *et al*., 2018). In the present study, a higher phylogenetic clustering degree in bacterial community of dinoflagellate dominant feeding group indicated a stronger host selectivity, which was suspected to be brought by an emergent adaptations of host-microbiome system under physiological stress of poor nutrition (Fan *et al*., 2012). Under a short-term physiological pressure, the host will adjust the size and structure composition of bacterial community stress-dependently, so as to form a heritable evolutionary adaptation and maximize survival rate of the host. After the rapid selection, closely related bacteria lineages will occur together and the host's immunity and resistance will be enhanced subsequently, which is also a high energy-intensive process (Kokou *et al*., 2018). The emergent adaption brought by host-microbiome system was supposed to help the system survive under the poor nutrition stress, but the energy consumption of the host in this process could not be underestimated, which made it more difficult for the oysters in dinoflagellate dominant feeding group to go through the physiological stress of poor nutrition. Phylogenetically clustering of bacterial community is also crucial to oysters under OsHV-1 viral infection. After being attacked by the virus, the host would lack selectivity and modification to the bacterial community, which indicates that random effects have a main influence on the assembly of bacterial community. Permissiveness of bacterial community has raised bacteremia and become an important cause of oyster mortality (Lorgeril *et al*., 2018).

In conclusion, the composition and structure of bacteria community in digestive glands of oysters fed with dinoflagellate dominant diet were significant different from that in the diatom dominant feeding group. Deficiency of important functional bacteria and insufficiency of bacteria metabolite led to the depressing physiological and metabolic conditions of oysters in dinoflagellate dominant feeding group. The bacterial community in dinoflagellate dominant feeding group suggested a potential immune system insensitivity to bacterial pathogens and a weak euryhaline adaptation capability of the host. The potential glucose consumption of the bacterial community in dinoflagellate dominant feeding group and a high energy-intensive emergent adaption to poor nutrition environment further brought with a more difficult survival challenge for oysters. Dinoflagellate dominant diet led to an imbalance status of the bacterial community in oyster digestive glands and the harmful impacts on hosts, which might be one of main reasons of oyster massive mortalities.

Acknowledgments

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RESEARCH REPORT- SUPPLEMENTARY MATERIAL

Comparative characterization of bacterial communities in digestive glands of *Crassostrea gigas* fed with different microalgal diets

S Han *et al*

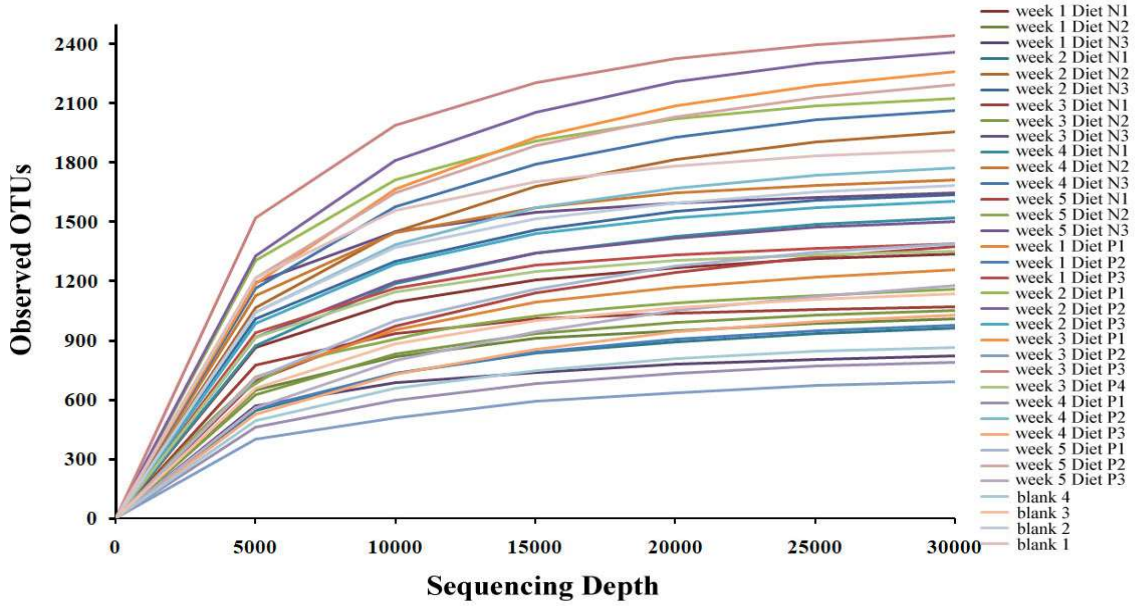

Fig. S1 Rarefaction curves of the partial 16S rRNA gene sequences from oyster digestive glands under different diets treating

Table S1 DNA concentration of all samples in the experiment

Sample name	DNA concentration (ng/μL)	OD260/280	Sample name	DNA concentration (ng/μL)	OD260/280
blank1	268.9	1.87	week5 Diet N3	S253.6	1.89
blank2	293.8	1.88	week1 Diet P1	262.0	1.87
blank3	319.4	1.91	week1 Diet P2	252.0	1.87
blank4	290.0	1.88	week1 Diet P3	232.0	1.87
week1 Diet N1	314.0	1.88	week2 Diet P1	14.9	1.67
week1 Diet N2	337.0	1.88	week2 Diet P2	86.6	1.88
week1 Diet N3	224.0	1.83	week2 Diet P3	242.8	1.84
week2 Diet N1	197.5	1.88	week3 Diet P1	230.4	1.93
week2 Diet N2	249.0	1.87	week3 Diet P2	251.8	1.87
week2 Diet N3	268.0	1.88	week3 Diet P3	262.2	1.88
week3 Diet N1	263.6	1.88	week3 Diet P4	267.7	1.88
week3 Diet N2	288.2	1.90	week4 Diet P1	254.2	1.87
week3 Diet N3	269.9	1.90	week4 Diet P2	254.2	1.88
week4 Diet N1	308.1	1.88	week4 Diet P3	251.3	1.87
week4 Diet N2	295.0	1.88	week5 Diet P1	279.3	1.87
week4 Diet N3	268.7	1.80	week5 Diet P2	253.7	1.87
week5 Diet N1	219.7	1.88	week5 Diet P3	249.8	1.88
week5 Diet N2	296.9	1.87			

Table S2 The sequence number of different samples after quality filtering

Sample name	Sequence number	Sample name	Sequence number
blank1	49340	week5 Diet N3	40114
blank2	38072	week1 Diet P1	48170
blank3	41845	week1 Diet P2	37763
blank4	39812	week1 Diet P3	41084
week1 Diet N1	41094	week2 Diet P1	40975
week1 Diet N2	44133	week2 Diet P2	36583
week1 Diet N3	50773	week2 Diet P3	42962
week2 Diet N1	37873	week3 Diet P1	40105
week2 Diet N2	32351	week3 Diet P2	42413
week2 Diet N3	32844	week3 Diet P3	35585
week3 Diet N1	37468	week3 Diet P4	37087
week3 Diet N2	42735	week4 Diet P1	37714
week3 Diet N3	44748	week4 Diet P2	36576
week4 Diet N1	45553	week4 Diet P3	40653
week4 Diet N2	41454	week5 Diet P1	39526
week4 Diet N3	44280	week5 Diet P2	40699
week5 Diet N1	43452	week5 Diet P3	41768
week5 Diet N2	45144		

Table S3 NSTI value of all samples in the PICRUSt analysis.

Sample name	Weighted NSTI	Sample name	Weighted NSTI
week5 Diet N1	0.161	week5 Diet P1	0.054
week5 Diet N2	0.247	week5 Diet P2	0.214
week5 Diet N3	0.265	week5 Diet P3	0.233