

Supplementary Materials

Title: The gill-associated microbiome is more affected by exogenous *Chlorella pyrenoidosa* addition than the microbiomes in the water and gut in the GIFT Tilapia (*Oreochromis niloticus*) aquaculture system

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Methods and materials

Bioinformatics

The paired-end raw sequences were processed with QIIME v1.9.1[1]. Firstly, we filtered sequences for all fastq files using the following criteria: (i) sequences having an average quality score < 25 and a read length < 200 bp were removed; (ii) reads having any mismatch with primer matching or comprising blurred characters were discarded; and (iii) sequences that could not be assembled were removed. The filtering on sequences was conducted using the command '*split_libraries_fastq.py*' and '*join_paired_ends.py*' of QIIME. UCHIME de novo strategy was used for identifying and removing chimeric sequences [2]. We then clustered operational taxonomic units (OTUs) using UCLUST with a 97% identity threshold [3]. The taxonomic information of each OTU was then explored based on SILVA database (Release138 <http://www.arb-silva.de>). Non-bacterial sequences were also removed by the command '*filter_taxa_from_otu_table.py*' for the subsequent analyses. To minimize sequencing errors, OTUs having reads < 15 (0.0005% of total reads) were discarded using the command '*filter_otus_from_otu_table.py*' [4]. Finally, samples were rarefied at 24,577 sequences according to the minimum sequence number.

References:

1. Caporaso, J.G.; Lauber, C.L.; Walters, W.A.; Berglyons, D.; Huntley, J.; Fierer, N.; Owens, S.M.; Betley, J.; Fraser, L.; Bauer, M. Ultra-high-throughput microbial community analysis on the Illumina HiSeq and MiSeq platforms. *The ISME Journal* **2012**, *6*, 1621-1624.
2. Edgar, R.C.; Haas, B.J.; Clemente, J.C.; Quince, C.; Knight, R. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics* **2011**, *27*, 2194-2200, doi:10.1093/bioinformatics/btr381.
3. Edgar, R.C. Search and clustering orders of magnitude faster than BLAST. *Bioinformatics* **2010**, *26*, 2460-2461, doi:10.1093/bioinformatics/btq461.
4. Bokulich, N.A.; Subramanian, S.; Faith, J.J.; Gevers, D.; Gordon, J.I.; Knight, R.; Mills, D.A.; Caporaso, J.G. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature Methods* **2013**, *10*, 57-U11, doi:10.1038/nmeth.2276.

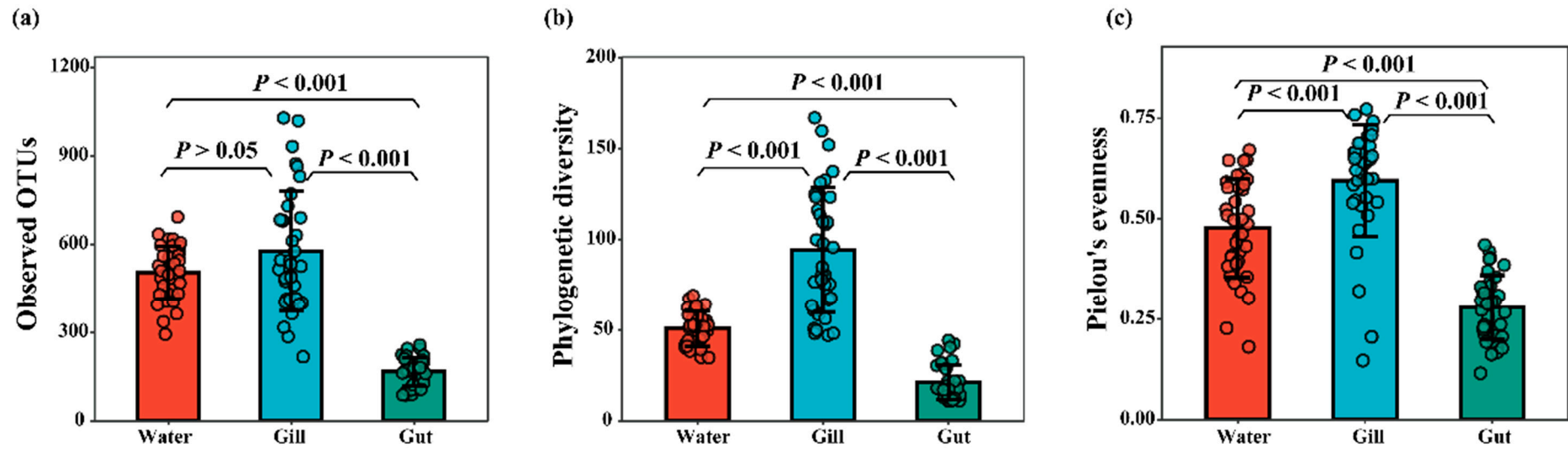


Figure S1. The comparisons in alpha diversity of bacterial communities in water, gill and gut. The samples from different treatments and timepoints were mixed. The significant differences in observed OTUs (a), phylogenetic diversity (b), and Pielou's evenness (c) of bacterial communities between different microhabitats (i.e., water, gill and gut) were tested by student's t tests. ns, $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

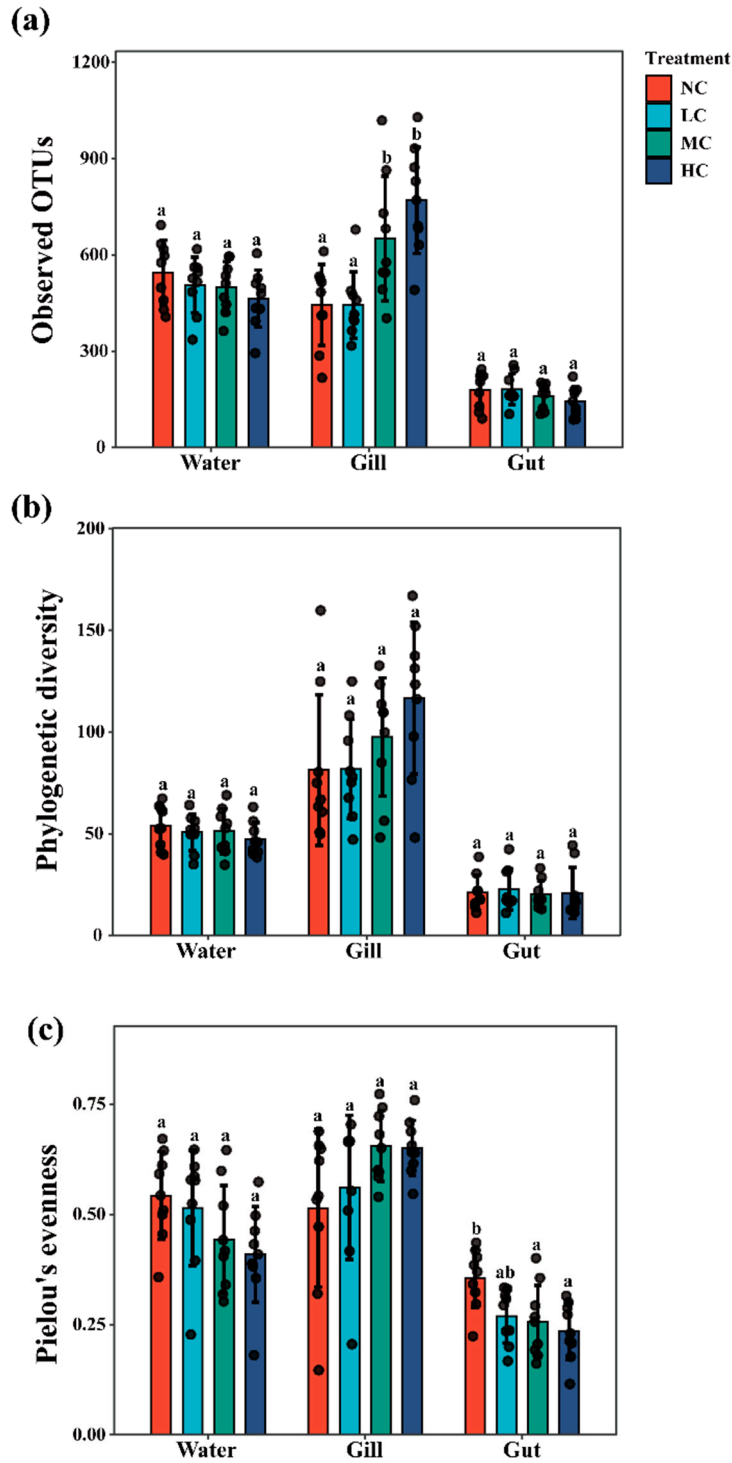


Figure S2. The comparisons of alpha diversity of bacterial communities between different treatments (different *Chlorella pyrenoidosa* additions) in water, gill and gut. The samples from different timepoints were mixed. The significant differences in observed OTUs (a), phylogenetic diversity (b), and Pielou's evenness (c) of bacterial communities between different microhabitats (i.e., water, gill and gut) indicated by different letters were tested by ANOVA tests followed by Turkey post hoc tests.

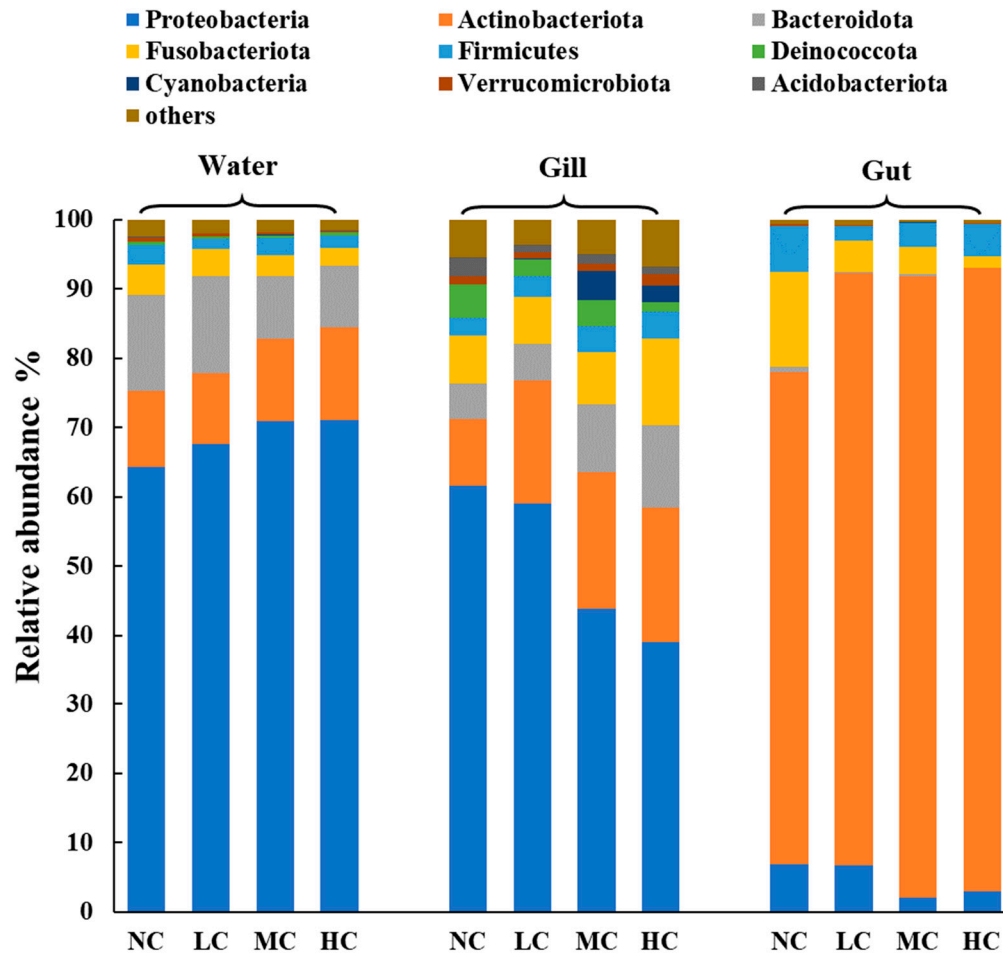


Figure S3. The relative abundance of the dominant phyla (relative abundance > 0.5%) of bacterial communities inhabiting different microhabitats in treatments with different *Chlorella pyrenoidosa* additions. NC, no addition; LC, low concentration; MC, medium concentration; HC, high concentration.

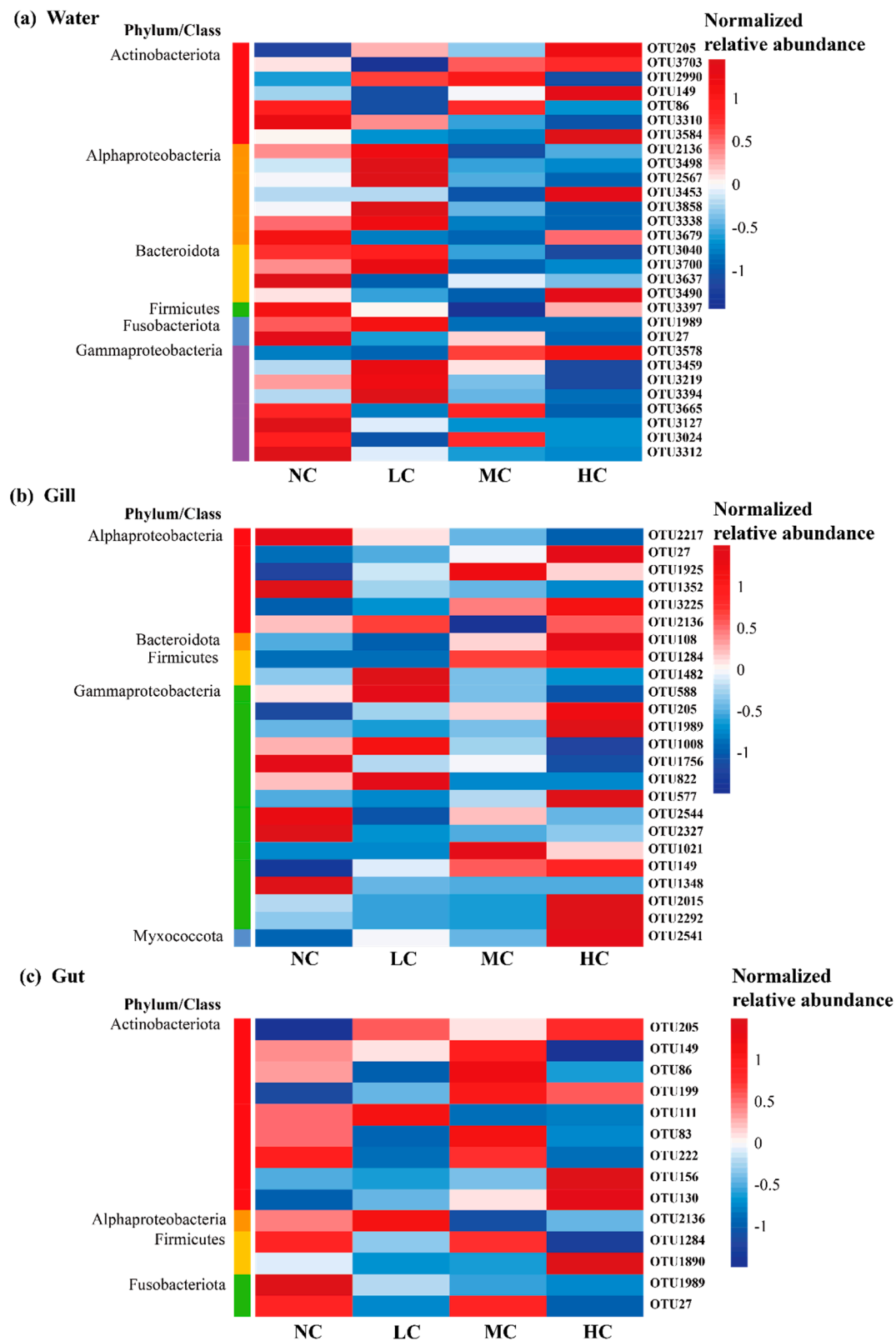
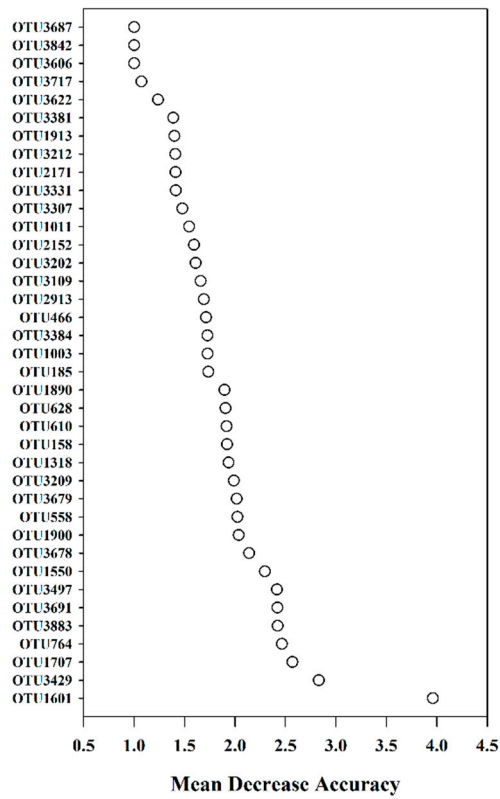
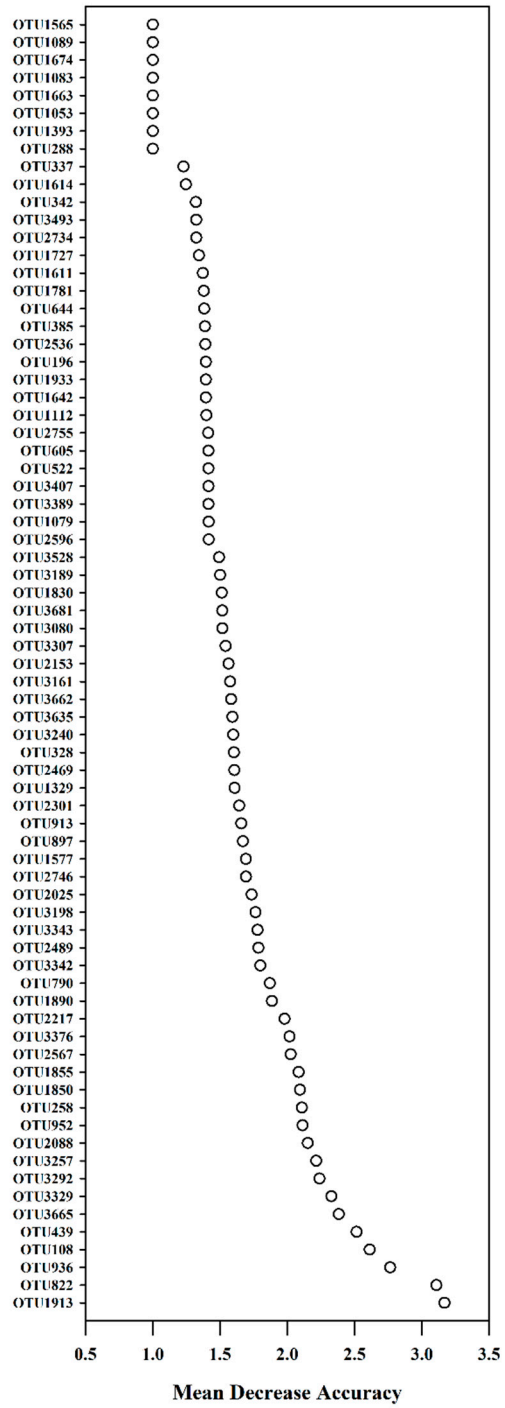


Figure S4. Heatmaps showing the relative abundance of dominant OTUs (mean relative abundance > 0.5%) across different treatments (different *Chlorella pyrenoidosa* additions) in water (a), gill (b) and gut (c).

(a) Water



(b) Gill



(c) Gut

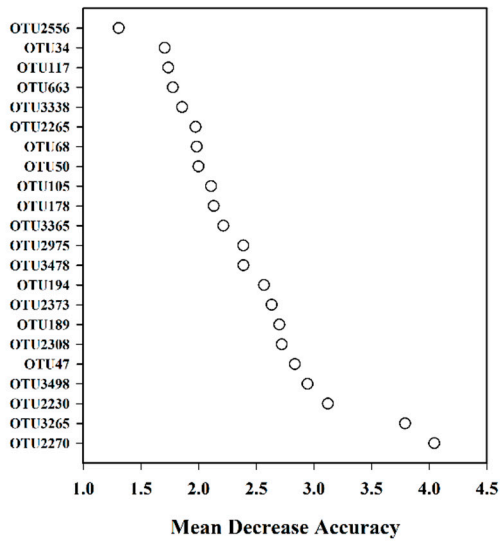


Figure S5. Bacterial taxa that are significantly influenced by the *Chlorella pyrenoidosa* addition in water, gill and gut detected by random forest models.