Supplementary Material

# Supplementary Tables

Table S1: Environmental parameters in seawater at Licun estuary and Zhanqiao Pier. Environmental parameters (mean ± standard error, n = 3) include temperature, salinity, dissolved oxygen and pH, which were measured by a Pro Plus handheld multiparameter meter.

|  |  |  |
| --- | --- | --- |
|  | Licun estuary | Zhanqiao Pier |
| Temperature (°C) | 16.2±0.1 | 16.5±0.5 |
| Salinity | 29.63±0.1 | 31.30±0.3 |
| Dissolved oxygen (%) | 104.2±0.5 | 123.0±1.0 |
| pH | 8.20±0.02 | 8.40±0.02 |

Table S2: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the photosynthetic parameters of diatoms and cyanobacteria in muddy and sandy sediments (Fv/Fm: maximum quantum yield; α: photosynthetic efficiency; rETRmax: relative electron transport rate. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Parameters | Sediments | Algae | df | F | p-value |
| Fv/Fm | Mud | Diatom | 2 | 100.4 | **< 0.001** |
|  |  | Cyanobacteria | 2 | 31.2 | **< 0.001** |
|  | Sand | Diatom | 2 | 21.9 | **0.002** |
|  |  | Cyanobacteria | 2 | 7.8 | **0.022** |
| α | Mud | Diatom | 2 | 49.6 | **< 0.001** |
|  |  | Cyanobacteria | 2 | 4.7 | 0.090 |
|  | Sand | Diatom | 2 | 7.7 | **0.043** |
|  |  | Cyanobacteria | 2 | 0.054 | 0.949 |
| rETRmax | Mud | Diatom | 2 | 20.8 | **0.002** |
|  |  | Cyanobacteria | 2 | 23.3 | **0.001** |
|  | Sand | Diatom | 2 | 7.3 | **0.024** |
|  |  | Cyanobacteria | 2 | 3.3 | 0.109 |

Table S3: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the net growth rates of MPB, microalgae (diatoms and cyanobacteria), bacteria and viruses in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| MPB | Mud | 2 | 2.11 | 0.202 |
|  | Sand | 2 | 2.16 | 0.197 |
| Diatoms | Mud | 2 | 0.432 | 0.668 |
|  | Sand | 2 | 0.651 | 0.555 |
| Cyanobacteria | Mud | 2 | 11.5 | **0.009** |
|  | Sand | 2 | 0.834 | 0.479 |
| Bacteria | Mud | 2 | 1.7 | 0.274 |
|  | Sand | 2 | 7.6 | **0.022** |
| Viruses | Mud | 2 | 2.1 | 0.239 |
|  | Sand | 2 | 1.4 | 0.323 |

Table S4: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the ratio of chlorophyll *a* to phaeophytin *a* (chl *a*/ phaeo *a*) and the biomass ratio of cyanobacteria to diatoms (cyanobacteria/ diatoms) in MPB for muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| chl *a*/ phaeo *a* | Mud | 2 | 2.21 | 0.191 |
|  | Sand | 2 | 0.155 | 0.860 |
| cyanobacteria/ | Mud | 2 | 0.37 | 0.705 |
| diatoms | Sand | 2 | 7.1 | **0.026** |

Table S5: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the relative abundance of bacterial phyla and orders in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| Proteobacteria | Mud | 2 | 1.2 | 0.362 |
|  | Sand | 2 | 38 | **< 0.001** |
| Bacteroidota | Mud | 2 | 13.2 | **0.006** |
|  | Sand | 2 | 19.8 | **0.002** |
| Firmicutes | Mud | 2 | 24.1 | **0.001** |
|  | Sand | 2 | 10.6 | **0.011** |
| Rhodobacterales | Mud | 2 | 0.85 | 0.473 |
|  | Sand | 2 | 29.6 | **< 0.001** |
| Alteromonadales | Mud | 2 | 3.1 | 0.116 |
|  | Sand | 2 | 132.0 | **< 0.001** |
| Flavobacteriales | Mud | 2 | 14.4 | **0.005** |
|  | Sand | 2 | 92.0 | **< 0.001** |
| Bacillales | Mud | 2 | 8.4 | **0.018** |
|  | Sand | 2 | 8.2 | **0.019** |
| Peptostreptococcales- | Mud | 2 | 122.6 | **< 0.001** |
| Tissierellales | Sand | 2 | 26.0 | **0.001** |

Table S6: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the relative abundance of eukaryotic phyla in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| Unclassified\_d\_Eukaryota | Mud | 2 | 13.2 | **0.006** |
|  | Sand | 2 | 0.07 | 0.937 |
| Rhodophyta | Mud | 2 | 1.1 | 0.379 |
|  | Sand | 2 | 0.1 | 0.902 |
| Bacillariophyta | Mud | 2 | 0.08 | 0.924 |
|  | Sand | 2 | 13.9 | **0.006** |
| Chlorophyta | Mud | 2 | 2.5 | 0.162 |
|  | Sand | 2 | 1.7 | 0.258 |
| Ciliophora | Mud | 2 | 3.8 | 0.086 |
|  | Sand | 2 | 43.7 | **< 0.001** |
| Nematoda | Mud | 2 | 1.6 | 0.273 |
|  | Sand | 2 | 2.9 | 0.134 |
| Arthropoda | Mud | 2 | 0.84 | 0.476 |
|  | Sand | 2 | NA | NA |
| Ascomycota | Mud | 2 | 2.4 | 0.171 |
|  | Sand | 2 | 2.3 | 0.184 |

Table S7: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the function abundance predicted by FAPROTAX in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| chemoheterotrophy | Mud | 2 | 221.0 | **< 0.001** |
|  | Sand | 2 | 361.7 | **< 0.001** |
| aerobic | Mud | 2 | 131.1 | **< 0.001** |
| chemoheterotrophy | Sand | 2 | 234.9 | **< 0.001** |
| hydrocarbon degradation | Mud | 2 | 25.8 | **0.001** |
|  | Sand | 2 | 282.2 | **< 0.001** |
| dark oxidation of | Mud | 2 | 0.20 | 0.822 |
| sulfur compounds | Sand | 2 | 4.9 | **0.047** |
| fermentation | Mud | 2 | 4.5 | 0.065 |
|  | Sand | 2 | 3.2 | 0.115 |
| nitrate reduction | Mud | 2 | 3.7 | 0.087 |
|  | Sand | 2 | 2.7 | 0.147 |
| respiration of sulfur | Mud | 2 | 32.2 | **< 0.001** |
| compounds | Sand | 2 | 29.9 | **< 0.001** |
| thiosulfate respiration | Mud | 2 | 36.3 | **< 0.001** |
|  | Sand | 2 | 30.6 | **< 0.001** |

Table S8: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the function abundance predicted by PICRUST2 in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| Carbohydrate metabolism | Mud | 2 | 0.83 | 0.479 |
|  | Sand | 2 | 0.20 | 0.822 |
| Starch and sucrose metabolism | Mud | 2 | 5.4 | **0.046** |
|  | Sand | 2 | 2.5 | 0.162 |
| Energy metabolism | Mud | 2 | 0.60 | 0.577 |
|  | Sand | 2 | 0.12 | 0.889 |
| Glycolysis/Gluconeogenesis | Mud | 2 | 0.402 | 0.686 |
|  | Sand | 2 | 0.06 | 0.939 |
| Citrate cycle | Mud | 2 | 0.50 | 0.632 |
|  | Sand | 2 | 0.07 | 0.934 |
| Oxidative phosphorylation | Mud | 2 | 0.62 | 0.572 |
|  | Sand | 2 | 0.02 | 0.976 |

Table S8: Results of one-way ANOVA tests showing the effect of different CO2 enhancements (400, 700 and 1000 ppm) on the function abundance predicted by PICRUST2 in muddy and sandy sediments. Significant difference (P < 0.05) indicated in bold.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Parameters | Sediments | df | F | p-value |
| Biofilm formation | Mud | 2 | 11.0 | **0.010** |
| *Vibrio cholerae* | Sand | 2 | 55.2 | **< 0.001** |
| Biofilm formation | Mud | 2 | 5.3 | **0.047** |
| *Escherichia coli* | Sand | 2 | 8.2 | **0.019** |
| Biofilm formation | Mud | 2 | 8.5 | **0.018** |
| *Pseudomonas aeruginosa* | Sand | 2 | 20.5 | **0.002** |
| Cell motility | Mud | 2 | 12.3 | **0.007** |
|  | Sand | 2 | 18.1 | **0.003** |
| Flagellar assembly | Mud | 2 | 10.5 | **0.011** |
|  | Sand | 2 | 12.6 | **0.007** |
| Bacterial chemotaxis | Mud | 2 | 14.8 | **0.005** |
|  | Sand | 2 | 29.8 | **< 0.001** |

Table S9: Changes in microphytobenthos chl *a* concentration of intertidal sandy and muddy sediments during a 28-day period under three different CO2 treatments. Chl *a* concentration (mean ± standard error, n = 3) was measured on day 0 and day 28 by a Turner Designs 10-AU Fluorometer, with a unit of mg chl *a* m-2. Three CO2 treatments are 400 ppm, 700 ppm and 1000 ppm.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sediment | Day | 400 | 700 | 1000 |
| Sand | 0 | 341.23±21.48 | 341.23±21.48 | 341.23±21.48 |
| Mud | 0 | 111.36±51.85 | 111.36±51.85 | 111.36±51.85 |
| Sand | 28 | 1057.11±150.47 | 796.21±166.13 | 579.86±151.19 |
| Mud | 28 | 414.41±10.52 | 180.56±62.00 | 260.90±42.69 |

Table S10: Changes in bacterial abundance of intertidal sandy and muddy sediments during a 28-day period under three different CO2 treatments. Bacterial abundance (mean ± standard error, n = 3, unit: x 109/L) was measured on day 0 and day 28 by a CytoFLEX Platform flow cytometer. Three CO2 treatments are 400 ppm, 700 ppm and 1000 ppm.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sediment | Day | 400 | 700 | 1000 |
| Sand | 0 | 9.08±1.28 | 9.08±1.28 | 9.08±1.28 |
| Mud | 0 | 23.51±2.22 | 23.51±2.22 | 23.51±2.22 |
| Sand | 28 | 168.64±46.60 | 105.51±1.02 | 40.85±8.10 |
| Mud | 28 | 149.92±33.04 | 94.72±12.56 | 72.40±25.05 |

Table S11: Changes in viral abundance of intertidal sandy and muddy sediments during a 28-day period under three different CO2 treatments. Viral abundance (mean ± standard error, n = 3, unit: x 1010/L) was measured on day 0 and day 28 by a CytoFLEX Platform flow cytometer. Three CO2 treatments are 400 ppm, 700 ppm and 1000 ppm.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Sediment | Day | 400 | 700 | 1000 |
| Sand | 0 | 6.34±1.17 | 6.34±1.17 | 6.34±1.17 |
| Mud | 0 | 3.82±1.23 | 3.82±1.23 | 3.82±1.23 |
| Sand | 28 | 115.69±17.75 | 87.90±13.38 | 114.97±14.45 |
| Mud | 28 | 40.65±7.53 | 80.46±14.14 | 54.12±4.91 |

Table S12: Topological features of co-occurrence networks of biotic communities in muddy and sandy intertidal sediments at different pCO2 incubation conditions. Network analysis was conducted in R studio and Gephi. OTUs with an average relative abundance >0.1% and strong correlations (Spearman’s rank correlation coefficient, r > 0.9 (or r < −0.9)) were selected to construct the networks.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | S400 | S700 | S1000 | M400 | M700 | M1000 |
| Number of nodes | 152 | 164 | 148 | 211 | 196 | 200 |
| Number of edges | 3293 | 3838 | 3143 | 5787 | 5731 | 6285 |
| Positive correlations | 52.84% | 54.82% | 58.86% | 60.65% | 60.43% | 63.23 |
| Negative correlations | 47.16% | 45.18% | 41.14% | 39.35% | 39.57% | 36.77 |
| Average degree | 43.329 | 46.805 | 42.473 | 54.853 | 58.48 | 62.85 |
| Network diameter | 1 | 1 | 1 | 1 | 1 | 1 |
| Network density | 0.287 | 0.287 | 0.289 | 0.261 | 0.3 | 0.316 |
| Modularity | 6.872 | 4.153 | 1.909 | 1.674 | 1.76 | 1.33 |
| Average clustering | 1 | 1 | 1 | 1 | 1 | 1 |
| coefficient |  |  |  |  |  |  |

# Supplementary Figures

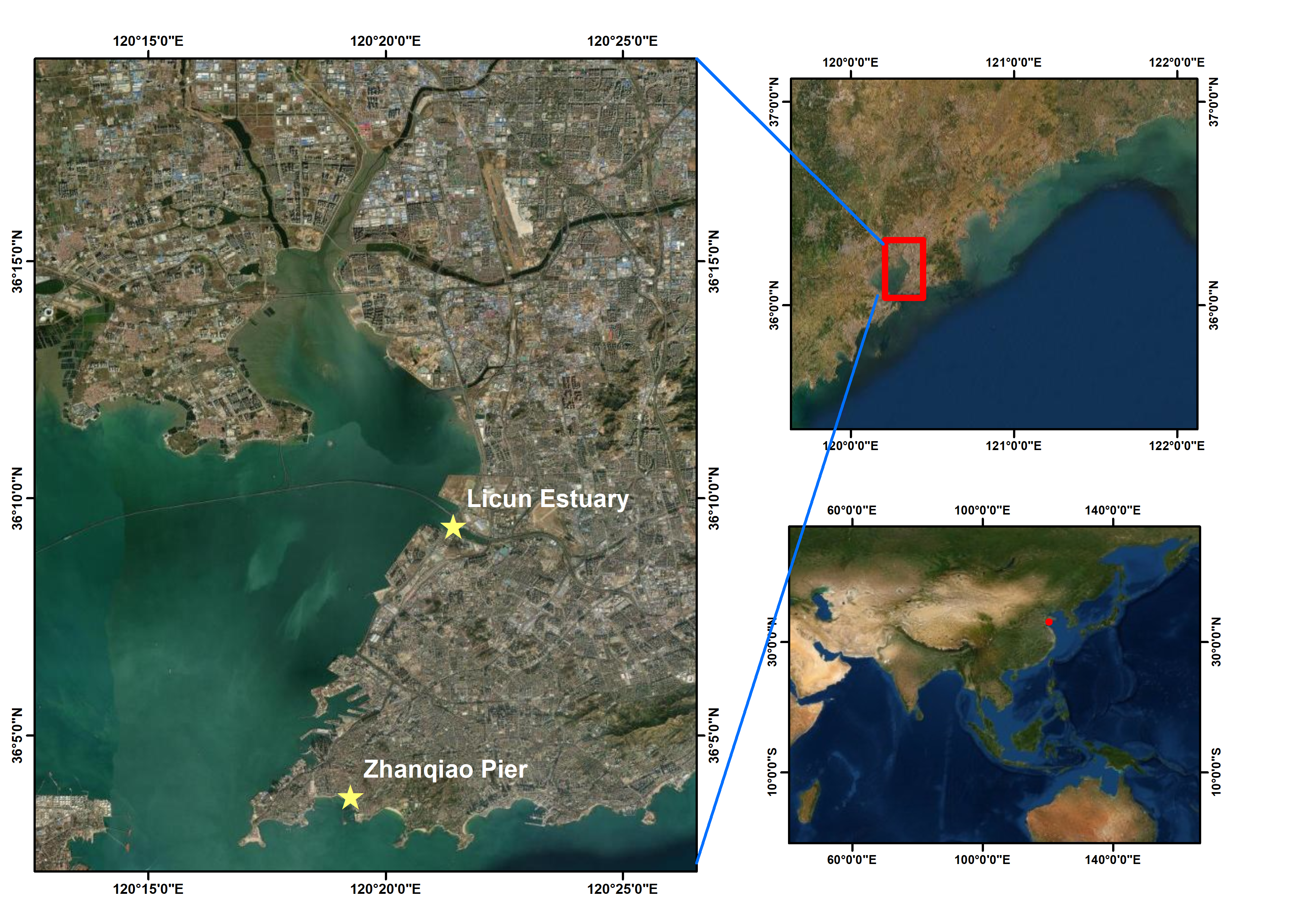
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Figure S1: Location of the sampling sites in the Qingdao area, China. Both sites are located in an area characterized by intense urban and industrial development. Muddy sediments were collected at Licun estuary and sandy sediments were collected at Zhanqiao Pier.



Figure S2: Effects of ocean acidification on the ratio of chlorophyll *a* to phaeophytin *a* in muddy and sandy intertidal sediments. Brown color represents sandy sediments, and blue color represents muddy sediments. Chlorophyll *a* and phaeophytin *a* concentration was measured by a Turner Designs 10-AU Fluorometer, with a unit of mg chl *a* m-2. Three CO2 treatments are 400 ppm, 700 ppm and 1000 ppm.



Figure S3: Effects of ocean acidification on the MPB community compositionin muddy and sandy intertidal sediments. Brown color represents sandy sediments, and blue color represents muddy sediments. Chl *a* concentration, as a proxy of biomass, was measured by a Phyto-PAM II Fluorometer. Three CO2 treatments are 400 ppm, 700 ppm and 1000 ppm.

3. Data Availability

Data submitted to and accepted by The National Center for Biotechnology Information (NCBI, Bethesda, Maryland USA)

Accession Sample Name SPUID Organism Tax ID BioProject  
SAMN31955048 Sample\_1 Sample\_1 marine sediment metagenome 412755 PRJNA907193  
SAMN31955049 Sample\_2 Sample\_2 marine sediment metagenome 412755 PRJNA907193  
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