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*Marine and Freshwater Research*

## Supplementary Material

# Organic matter and metal loadings influence the spatial gradient of the benthic bacterial community in a temperate estuary

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**Supplementary materials and methods**

At occasions, water quality and genomic data were taken two to three days apart. The implications of the sample disparity are minimal for our study. The water quality data is not expected to have changed on this small-time scale (especially as no major weather events occurred between the water quality and sediment sampling). In addition, the microbial community (especially DNA) would also not be expected to change that fast. Lastly, our findings and results present monthly dynamics, hence the time disparity of days will have a minimal to no effect.

Samples for physico-biochemical water column characteristics were analysed at Analytical Services Tasmania (AST; https://analyticalservices.tas.gov.au/). Methods for these analyses and contact details are outlined below.

**Contact details for AST Method Descriptions for the CSIRO December 2020**

Analytical Services Tasmania

18 St Johns Avenue, New Town, Tas. 7008, Australia

Phone: (03) 6165 3300

Email: alison.featherstone@ast.tas.gov.au

Web: http://analyticalservices.tas.gov.au

All the methods described below are included in AST's scope of NATA accreditation.

*Dissolved nutrients*

Samples are preserved by filtration through a 0.45-µm filter and freezing at –20°C.

*Ammonia, in-house method 1205*

Analysis is performed using a Lachat Flow Injection analyser. This method is based on APHA Standard Method 4500-NH3-H (APHA 2005).

The ammonia determination is based on the Berthelot reaction. Ammonia reacts in alkaline solution with hypochlorite to form monochloramine at a pH between 8 and 11.5 which, in the presence of phenol, catalytic amounts of nitroprusside (nitroferricyanide) and excess hypochlorite, forms indophenol blue absorbing at 630 nm.

Results are reported as milligrams per litre of N, with a method reporting limit of 0.005 mg $L^{-1}$.

*Nitrate and nitrite, in-house method 1205*

Analysis is performed using a Lachat Flow Injection analyser. This method is based on APHA Standard Method 4500-NO3-I (APHA 2005).

The nitrate and nitrite determination involves the nitrate being quantitatively reduced to nitrite by passage of the sample through a copperised cadmium column at a pH of 8. The $NO_x$ (reduced nitrate plus original nitrite),

is then determined by diazotization with sulphanilamide under acidic conditions to form a diazonium ion which is coupled with N-(1-Napthyl) Ethylenediamine Dihydrochloride, (NEDD). The resulting pink dye absorbs at 520 nm.

Results are reported as milligrams per litre of N, with a method reporting limit of 0.002 mg $L^{-1}$.

*Dissolved reactive phosphorus, in-house method 1205*

Analysis is performed using a Lachat Flow Injection analyser. This method is based on APHA Standard Method 4500-P G (APHA 2005).

The phosphate determination involves the orthophosphate ion ($PO_4^{3-}$) reacting with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This complex is reduced with ascorbic acid to form a blue complex which absorbs at 880 nm.

Results are reported as milligrams per litre of P, with a method reporting limit of 0.003 mg $L^{-1}$.

**Total nutrients**

Samples are preserved by freezing at –20°C.

*Total nitrogen, in-house method 1206*

Analysis is performed using a Lachat Flow Injection analyser. This method is based on APHA Standard Method 4500-Norg D (APHA 2005). Total Kjeldahl Nitrogen (TKN) present in the sample is determined by converting the nitrogen to ammonium sulphate in a sulphuric acid-potassium sulphate digestion and includes organic nitrogen and ammonia. This converts amino-nitrogen of many organic materials, free ammonia, and ammonium nitrogen to ammonium sulphate. Oxides of nitrogen are not converted to ammonium sulphate by this digestion. Kjeldahl Nitrogen is analysed by reacting the digested sample with an alkaline buffer, salicylate and hypochlorite to form a blue coloured compound. The colour developed is intensified by nitroprusside and absorbs at 660 nm. The absorbance is proportional to the concentration of TKN in the original sample. EDTA is present in the buffer to prevent precipitation of calcium and magnesium. Total Nitrogen is calculated by adding the TKN and oxides of nitrogen (nitrate and nitrite) determined from in-house method 1205 described above. Results are reported as milligrams per litre of N, with a method reporting limit of 0.1 mg $L^{-1}$.

*Total Phosphorus, in-house method 1206*

Analysis is performed using a Lachat Flow Injection analyser. This method was developed by Lachat Instruments and is a combination of 'Quikchem' Method 10-115-01-1-C and Method 10-107-06-2-E. The chemistry is also based on APHA Standard Method 4500-P G (APHA 2005). Total Phosphorus includes orthophosphates, condensed phosphates (pyro-, meta-, and other polyphosphates), and organically bound phosphates. They occur in solution, in particles or detritus, and in the bodies of aquatic organisms. Total Phosphorus (TP) present in the sample is determined by converting the phosphorus compounds to orthophosphate in a sulfuric acid–potassium sulphate digestion. TP is analysed by reacting the digested sample with ammonium molybdate and antimony potassium tartrate under acidic conditions to form a complex. This

complex is reduced with ascorbic acid to form an intense blue complex which absorbs at 880 nm. The absorbance is proportional to the concentration of Total Phosphorus in the original sample. Results are reported as milligrams per litre of P with a method reporting limit of 0.01 mg $L^{-1}$.

**NPOC (non-purgeable organic carbon), carbon in water by wet oxidation, in-house method 1210**

This method follows 'USEPA Method 9060A, *Total Organic Carbon'* and 'Standard Methods for the Examination of Water and Wastewater. 22nd Ed, 2012. American Public Health Association. 5310 A and C Total Organic Carbon' and OI Analytical application note 32171209, *TOC Analysis of Saltwater Matrices Using Heated Sodium Persulfate Oxidation*.

Samples requiring dissolved non-purgeable organic carbon are filtered through a 0.45 µm filter. Samples are preserved by acidification with phosphoric acid to 1% and refrigeration.

Samples are analysed using an O.I Analytical Aurora 1030 Wet Oxidation TOC Analyzer.

The sample is first reacted with phosphoric acid and purged with an inert gas to remove inorganic forms of carbon. Then sodium persulfate is added and the sample heated, converting organic carbon to carbon dioxide ($CO_2$). $CO_2$ is purged from the sample and measured directly by a non-dispersive infrared detector. The amount of $CO_2$ produced is directly proportional to the concentration of carbonaceous material in the sample.

Reference material is Inorganic Ventures TOCKHP1 traceable to NIST SRM 84L Lot # 84L (accredited to ISO 17034 General Requirements for the Competence of Reference Material Producers), zero sample is Milli-Q water and nicotinic acid is used as matrix additions to evaluate oxidation efficiency every 20 samples. The Method Reporting Limit is 0.3 mg $L^{-1}$.

**Metals in saline water by ICP-AES, method 1315**

In-house method based upon APHA Methods 3030, 3120, 3120 B (APHA 2005) and USEPA 200.7. Samples are acidified to 1% nitric acid and left for at least 16 h. After this time a 40-mL sub-sample is digested for 2 h in a 10% hot nitric acid matrix at 90 ± 5°C. Samples are made back up to 40-mL volume using water with a resistivity of 18.2 MΩ and analysed using an Agilent 730-ES ICP-AES. Element concentration in samples is determined from a calibration curve established from standards of known element concentration. Standards are prepared in a high salt matrix through the addition of high purity NaCl to give a final concentration of 30 g $L^{-1}$. Samples are introduced into the plasma through a concentric nebuliser and cyclonic spray chamber.

**Chlorophyll *a*, in-house method 1602**

This is an in-house method based on the American Public Health Association, Standard Methods for the Examination of Water and Wastewater, 22nd edition, 2012. 10200 H Chlorophyll. Samples are kept dark and filtered under vacuum through a glass-fibre filter immediately upon receipt (or as soon as is practical) and frozen (–20°C) until analysis. The sample is extracted by adding a buffered 90% acetone solution and ultrasonication in an ice bath for 20 min, followed by further extraction in the freezer. Samples are then centrifuged and the absorbance read using a Varian Cary 50 UV-visible spectrophotometer. Chlorophyll-*a* (µg $mL^{-1}$) is calculated according to the calculation in APHA. Method reporting limit is 0.5 mg $m^{-3}$.

Calculation from APHA:

$$\text{Chlorophyll-}a = 11.85(OD664-OD750) - 1.54(OD647-OD750) - 0.08(OD630-OD750)$$

where OD664, OD647, OD630 and OD750 are the optical densities (with a 1-cm light path) at the respective wavelengths. OD750 is used to correct for turbidity. The final value is calculated accounting for the volume of sample filtered and extraction volume of buffered acetone:

$$\text{Chlorophyll-}a = \text{Chl-}a \times \text{extract volume} \div \text{sample volume} \times \text{Dilution Factor}$$

where chlorophyll-*a* (mg $m^{-3}$), Chl-*a* (µg $mL^{-1}$), extract volume (mL), and sample volume (L)

*Metals and stable isotope analyses*

Metal concentrations including phosphorus (dry weight) were analysed at Analytical Services Tasmania (AST; https://analyticalservices.tas.gov.au/). Metals were analysed by inductively coupled plasma–atomic emission spectroscopy (ICP-AES; method 2301). Sediment samples were dried at 104°C and ground to particle size <2 mm. A subsample was then digested in hot aqua regia (mixture of hydrochloric and nitric acids). The digests were passed through a 0.45-mm syringe filter before analysis. Samples were introduced to the ICP-AES by nebulisation and each element in solution emits light of specific and characteristic wavelengths from the plasma (~10 000K). Element concentrations were determined from a calibration curve established from standards of known element concentrations. Results are reported on a dry matter basis (DMB). Sediment mercury analyses were done by cold vapour atomic fluorescence spectroscopy (CV-AFS). Moisture content of the sample was determined in a separate measurement. A wet soil sample was digested in a hot nitric and sulfuric acid solution. The digestion process oxidises the forms of mercury to $Hg^{2+}$. The sample was then introduced to the CV-AFS and the $Hg^{2+}$ was reduced on-line by reaction with $SnCl_2$ to form an elemental mercury vapour which is detected by atomic fluorescence spectrometry. The measured signal was compared to a calibration curve established from standards of known mercury concentration. The mercury content in the wet soil sample was then back calculated using the determined moisture content value to report the mercury in soil content on a dry matter basis (DMB).

The isotopic composition of the total N and organic C in the sediments ($\delta^{15}N$ and $\delta^{13}C$) were analysed at the CSIRO laboratories in Hobart, Tasmania. Sediments were freeze-dried until dry, ~48 h. Sediment samples analysed for carbon were weighed into aluminium cups (0.7–2.5 mg) Elemental Microanalysis Ltd, UK) and decalcified using 5% (m/v) sulfurous acid, while sediments analysed for nitrogen were weighed into tin cups (20–50 mg; Elemental Microanalysis Ltd, UK), according to the method of Verardo *et al*. (1990). Samples were analysed for nitrogen and carbon contents (both as percent dry weight), $\delta^{15}N$ relative to atmospheric $N_2$, and $\delta^{13}C$ relative to VPDB (Vienne Pee Dee Belemnite) using a Carlo Erba NA1500 CNS analyser interfaced with a Conflo IV to a Thermo Scientific Delta V Plus isotope ratio mass spectrometer operating in the continuous flow mode. Combustion and oxidation were achieved at 1020°C and reduction at 650°C respectively. Percent nitrogen and organic carbon were determined by comparison of instrument response (area) calibrated using standards with known carbon and nitrogen content. Stable isotope ratios were normalised with NBS-22, NBS-19, and LSVEC for carbon; and USGS-34, USGS-35, and IAEA-N3 for nitrogen. Instrument reproducibility

was 0.2‰ for carbon and 0.3‰ for nitrogen, sample reproducibility varied between 0 and 1.5‰. Results are presented in standard δ notation.

*Functional composition from* 16S *RNA*

The workflow for clustering the ASV sequences at the 97% similarity threshold can be found on https://github.com/EricRaes/marker_gene_manuscript

**Supplementary figures**

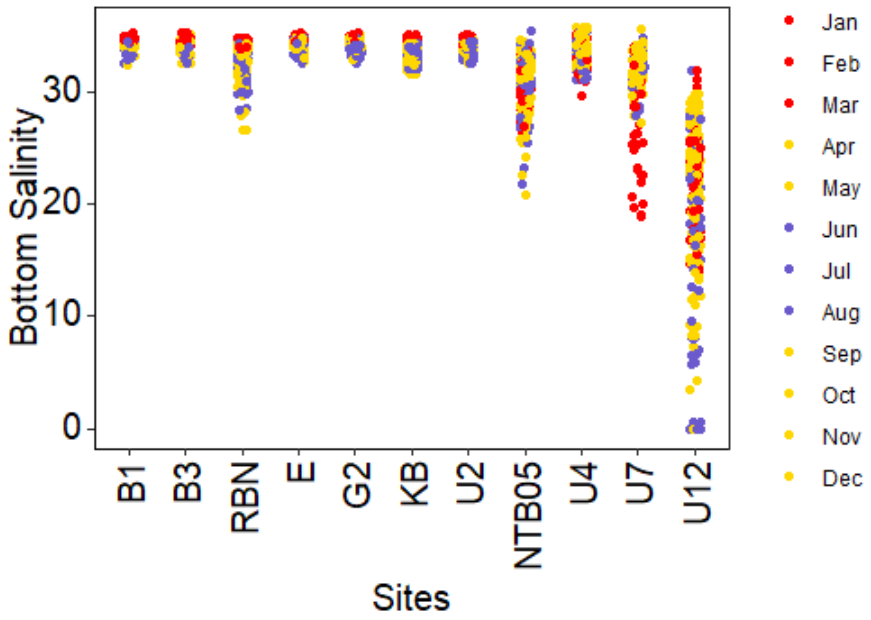


**Figure S1.** Bottom water salinity measurements collected over a decade in the Derwent estuary, Tasmania, from January 2010 until April 2020.

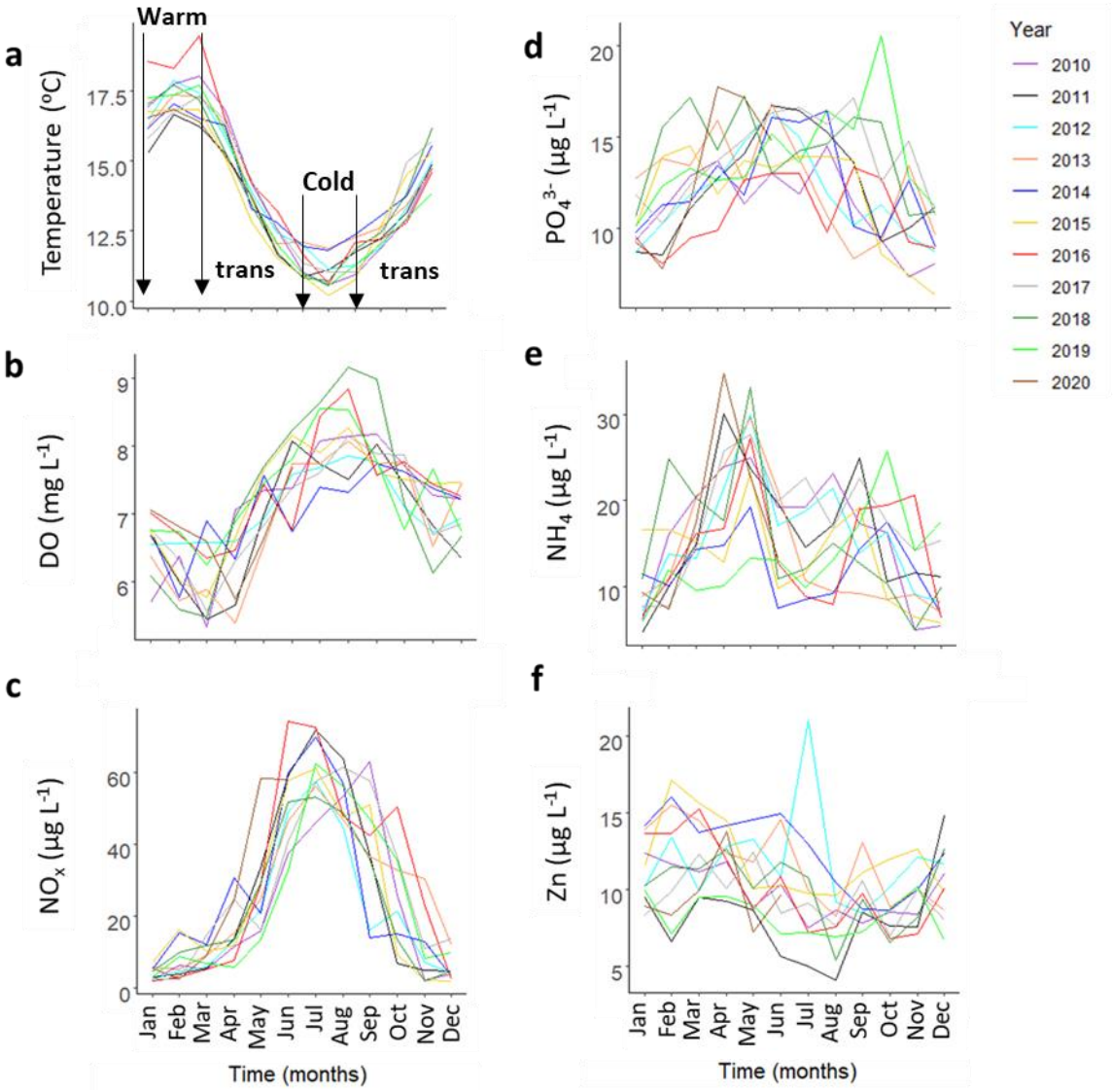


**Figure S2.** Physical and chemical bottom water measurements collected over a decade for 11 sites in the Derwent estuary, Tasmania (Fig. 1a–b) from January 2010 until April 2020. Data are monthly averaged for the 11 sites shown in Fig. 1a–b and span the lower and upper estuary. Bottom water (*a*) temperature (°C; $n$ = 3102;); (*b*) concentrations for dissolved oxygen (DO, mg $L^{-1}$; $n$ = 2982;); (*c*) nitrate and nitrate ($NO_x$, µg $L^{-1}$; $n$ = 1998)), (*d*) inorganic phosphorus ($PO_4^{3-}$, µg $L^{-1}$; $n$ = 1336)); (*e*) ammonium ($NH_4^+$, µg $L^{-1}$; $n$ = 1336); and (*f*)) zinc concentrations (Zn, µg $L^{-1}$; $n$ = 1286). Warmer (warm) and colder (cold) months along with the intermediate months (inter) are shown on (a), Supplementary Fig. S1 and Fig. S2.

## Supplementary tables

**Table S1. Site locations, sediment wet weight (g) for gDNA extraction, and gDNA concentrations (ng $\mu L^{-1}$).**

| BPA number | Site Derwent | water column depth (m) | Latitude (decimal) | Longitude (decimal) | Sediment weight (g) | Nanodrop (rep1 ng/uL) | Nanodrop (rep2 ng/uL) | Nanodrop (rep3 ng/uL) | average concentration (ng/ul) | Standard deviation (ng/ul) |
|---|---|---|---|---|---|---|---|---|---|---|
| 62718 | B1 | 16 | -43.01849 | 147.3414 | 0.27 | 8.438 | 7.643 | 8.252 | 8.1 | 0.42 |
| 62719 | B3 | 22 | -43.02182 | 147.378 | 0.25 | 12.98 | 9.991 | 9.591 | 10.9 | 1.85 |
| 62720 | RNB | 6 | -42.92349 | 147.4486 | 0.27 | 10.34 | 9.998 | 11.2 | 10.5 | 0.62 |
| 62721 | E | 25 | -42.91779 | 147.3802 | 0.27 | 18.01 | 18.5 | 14.56 | 17.0 | 2.15 |
| 62722 | G2 | 20 | -42.89099 | 147.3505 | 0.26 | 16.77 | 18.44 | 17.33 | 17.5 | 0.85 |
| 62723 | KB | 4 | -42.8745 | 147.3612 | 0.25 | 10.72 | 17.25 | 10.54 | 12.8 | 3.82 |
| 62724 | NTB5 | 8 | -42.84007 | 147.323 | 0.26 | 12.91 | 18.17 | 12.98 | 14.7 | 3.02 |
| 62727 | U2 | 27 | -42.85116 | 147.3369 | 0.25 | 18.17 | 18.9 | 21.6 | 19.6 | 1.81 |
| 62728 | U4 | 8 | -42.82398 | 147.3133 | 0.25 | 33.32 | 25.54 | 23.87 | 27.6 | 5.04 |
| 62729 | U7 | 10 | -42.78833 | 147.28638 | 0.25 | 16.12 | 12.37 | 12.41 | 13.6 | 2.15 |
| 62730 | U12 | 5 | -42.74416 | 147.22972 | 0.26 | 12.94 | 12.34 | 10.2 | 11.8 | 1.44 |

Nanodrop replicates are pseudo-replicates (i.e. measurements from the same sample).

**Table S2. Average sediment metal concentrations (mg $kg^{-1}$ dry matter basis, DMB) from 2018 to 2020.**

| | Arsenic (As) | | Cadmium (Cd) | | Copper (Cu) | | Iron (Fe) | | Lead (Pb) | | Mercury (Hg) | | Phosphorus ($PO_4^{3-}$) | | Zinc (Zn) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Estuary (Site)** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** | **mean** | **±sd** |
| Lower (B1; n=16) | 6.3 | 0.5 | 0 | 0 | 5 | 1 | 9227 | 302 | 25 | 2 | **0.2** | 0 | 294 | 17 | 69 | 8 |
| Lower (B3; n=26) | 10.1 | 0.9 | 0 | 0 | 3 | 1 | 8065 | 545 | 32 | 4 | **0.1** | 0 | 347 | 25 | 63 | 13 |
| Lower (RBN; n=26) | 3.9 | 0.5 | 0.1 | 0.2 | 7 | 1 | 6237 | 565 | **61** | 4 | **0.9** | 0.2 | 134 | 13 | 101 | 11 |
| Mid (E; n=26) | **19.4** | 2 | **3.9** | 0.2 | **73** | 5 | 39735 | 1742 | **434** | 28 | **7.7** | 1.9 | 885 | 45 | **1155** | 60 |
| Mid (G2; n=16) | **26.4** | 2.9 | **6.9** | 0.4 | **109** | 5 | 42075 | 2790 | **572** | 35 | **9.9** | 3.2 | 985 | 110 | **1725** | 75 |
| Mid (KB; n=15) | **41.3** | 2.7 | **10** | 0.6 | **146** | 6 | 42367 | 1986 | **686** | 40 | **13.2** | 1.5 | 832 | 69 | **2311** | 104 |
| Zinc-Refinery (U2; n=27) | **80.1** | 17 | **15.3** | 4.7 | **177** | 25 | 46375 | 3206 | **986** | 80 | **11.4** | 1.9 | 1413 | 198 | **2727** | 464 |
| Zinc-Refinery (NTB5; n=3) | **460.7** | 6.8 | **272.3** | 24.7 | **743** | 21 | 64167 | 907 | **2903** | 321 | **41** | 9.1 | 2446 | 37 | **25800** | 1778 |
| Upper (U4; n=4) | **118.3** | 5.1 | **16.8** | 0.5 | **172** | 4 | 44300 | 2252 | **760** | 57 | **7.7** | 1.2 | 2043 | 100 | **2453** | 25 |
| Upper (U7; n=12) | **39.9** | 4.9 | **10** | 1.5 | **79** | 12 | 22955 | 3210 | **382** | 43 | **4.1** | 0.9 | 576 | 135 | **1128** | 149 |
| Upper (U12; n=11) | 10 | 4 | 0.3 | 0.3 | 12 | 3 | 9451 | 4256 | **58** | 12 | **0.4** | 0.1 | 232 | 46 | 114 | 21 |
| **SQGV** | 20 | | 1.5 | | 65 | | NA | | 50 | | 0.15 | | NA | | 200 | |
| **SQGV-H** | 70 | | 10 | | 270 | | NA | | 220 | | 1 | | NA | | 410 | |

Sediment metal concentrations exceeding the quality guideline values (SQGV) are shown in red. The SQGV are shown at the bottom of the table. SQGV, lower sediment quality guideline value; SQGV-H, upper sediment quality guideline value for Australian and New Zealand estuaries (ARMCANZ, 2000). NA, no sediment quality guideline values.

**Table S3. ANOSIM results between 11 sites for *16S* rRNA gene data (ASVs).**

Tests for differences between unordered Site groups

**Global Test**

**Sample statistic (R): 0.893**

Significance level of sample statistic: 0.001

Number of permutations: 999 (Random sample from a large number)

**Pairwise Tests**

| Groups | R-value | Significance |
|---|---|---|
| B3-B1 | 0.076 | 0.036 |
| B3-RBN | 0.98 | 0.001 |
| B3-E | 0.987 | 0.001 |
| B3-G2 | 0.975 | 0.001 |
| B3-KB | 0.987 | 0.001 |
| B3-U2 | 0.988 | 0.001 |
| B3-U7 | 0.99 | 0.001 |
| B3-U12 | 0.99 | 0.001 |
| B3-U4 | 0.956 | 0.001 |
| B3-NTB5 | 0.958 | 0.003 |
| B1-RBN | 0.985 | 0.001 |
| B1-E | 0.99 | 0.001 |
| B1-G2 | 0.981 | 0.001 |
| B1-KB | 0.989 | 0.001 |
| B1-U2 | 0.99 | 0.001 |
| B1-U7 | 0.992 | 0.001 |
| B1-U12 | 0.992 | 0.001 |
| B1-U4 | 0.966 | 0.001 |
| B1-NTB5 | 0.967 | 0.001 |
| RBN-E | 0.96 | 0.001 |
| RBN-G2 | 0.887 | 0.001 |
| RBN-KB | 0.95 | 0.001 |
| RBN-U2 | 0.984 | 0.001 |
| RBN-U7 | 0.989 | 0.001 |
| RBN-U12 | 0.992 | 0.001 |
| RBN-U4 | 0.961 | 0.001 |
| RBN-NTB5 | 0.964 | 0.002 |
| E-G2 | 0.487 | 0.001 |
| E-KB | 0.92 | 0.001 |
| E-U2 | 0.973 | 0.001 |
| E-U7 | 0.991 | 0.001 |
| E-U12 | 0.992 | 0.001 |
| E-U4 | 0.955 | 0.001 |
| E-NTB5 | 0.964 | 0.001 |
| G2-KB | 0.463 | 0.001 |
| G2-U2 | 0.712 | 0.001 |
| G2-U7 | 0.976 | 0.001 |
| G2-U12 | 0.988 | 0.001 |
| G2-U4 | 0.789 | 0.002 |
| G2-NTB5 | 0.912 | 0.001 |
| KB-U2 | 0.789 | 0.001 |
| KB-U7 | 0.975 | 0.001 |
| KB-U12 | 0.991 | 0.001 |
| KB-U4 | 0.815 | 0.002 |
| KB-NTB5 | 0.944 | 0.002 |
| U2-U7 | 0.924 | 0.001 |
| U2-U12 | 0.984 | 0.001 |
| U2-U4 | 0.488 | 0.008 |
| U2-NTB5 | 0.866 | 0.001 |
| U7-U12 | 0.63 | 0.001 |
| U7-U4 | 0.432 | 0.020 |
| U7-NTB5 | 0.661 | 0.002 |
| U12-U4 | 0.914 | 0.002 |
| U12-NTB5 | 0.914 | 0.002 |
| U4-NTB5 | 0.704 | 0.10 |

Count data were centre log-transformed (CLR) transformed before analyses with an Aitchison (Euclidean) distance matrix.

**Table S4. ANOSIM results between 4 estuary zones for 16S rRNA gene data (ASVs).**

**Tests for differences between unordered Estuary groups**

**Global Test**

**Sample statistic (R): 0.840**

Significance level of sample statistic: 0.001

Number of permutations: 999 (Random sample from a large number)

**Pairwise Tests**

| Groups | R-value | Significance Level |
|---|---|---|
| Lower Estuary - Mid Estuary | 0.892 | 0.001 |
| Lower Estuary - Zinc Factory | 0.976 | 0.001 |
| Lower Estuary - Upper Estuary | 0.987 | 0.001 |
| Mid Estuary - Zinc Factory | 0.482 | 0.001 |
| Mid Estuary - Upper Estuary | 0.948 | 0.001 |
| Zinc Factory - Upper Estuary | 0.81 | 0.001 |

Count data were CLR transformed before analyses with an Aitchison (Euclidean) distance matrix.

**Table S5. Metadata correlations with bacterial community composition (CLR transformed).**

| Parameter | PC1 | PC2 | r2 | p-value |
|---|---|---|---|---|
| **TN** | -0.5631 | 0.82639 | 0.6537 | 0.001 |
| **d13C** | 0.99886 | 0.04767 | 0.5954 | 0.001 |
| **TOC** | -0.84698 | 0.53163 | 0.5813 | 0.001 |
| **Fe** | -0.48796 | 0.87287 | 0.5766 | 0.001 |
| **Sal_Bot** | 0.74294 | 0.66936 | 0.4776 | 0.001 |
| **TOC_bot** | -0.76738 | -0.6412 | 0.4114 | 0.001 |
| **PO4_dry** | -0.66525 | 0.74662 | 0.3144 | 0.001 |
| **Hg** | -0.58413 | 0.81166 | 0.2879 | 0.001 |
| **C.N.ratio** | -0.79442 | -0.60737 | 0.2789 | 0.001 |
| **Pb** | -0.7682 | 0.64022 | 0.2667 | 0.001 |
| **d15N** | 0.75558 | 0.65506 | 0.2582 | 0.001 |
| **O2_bot** | 0.05186 | -0.99865 | 0.193 | 0.001 |
| **NH4_bot** | -0.9995 | 0.03147 | 0.1912 | 0.001 |
| **Cu** | -0.8432 | 0.5376 | 0.1861 | 0.001 |
| **Chla_SFC** | -0.37816 | 0.92574 | 0.1223 | 0.002 |
| **PO4_bot** | -0.5588 | 0.8293 | 0.1181 | 0.002 |
| **As** | -0.99612 | 0.08796 | 0.1001 | 0.003 |
| **Zn** | -0.95523 | 0.29588 | 0.0653 | 0.016 |
| **Cd** | -0.99411 | -0.10835 | 0.0463 | 0.051 |
| **TN_bot** | -0.99882 | 0.04861 | 0.0224 | 0.308 |
| **Nox_bot** | -0.99287 | -0.11919 | 0.0135 | 0.474 |
| **T_Bot** | 0.42415 | 0.90559 | 0.0117 | 0.54 |

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Permutation: free

Number of permutations: 999

Red data are non-significant parameters.

**Table S6. ANOSIM results between 11 sites for PICRUSt2 data.**

Tests for differences between unordered Site groups

**Global Test**

**Sample statistic (R): 0.430**

**Significance level of sample statistic: 0.001**

Number of permutations: 999 (Random sample from a large number)

**Pairwise Tests**

| Groups | R-value | Significance |
|---|---|---|
| B3 - B1 | 0.036 | 0.123 |
| B3 - RBN | 0.558 | 0.001 |
| B3 - E | 0.505 | 0.001 |
| B3 - G2 | 0.485 | 0.001 |
| B3 - KB | 0.541 | 0.001 |
| B3 - U2 | 0.616 | 0.001 |
| B3 - U7 | 0.579 | 0.001 |
| B3 - U12 | 0.782 | 0.001 |
| B3 - U4 | 0.602 | 0.005 |
| B3 - NTB5 | 0.848 | 0.002 |
| B1 - RBN | 0.318 | 0.001 |
| B1 - E | 0.381 | 0.001 |
| B1 - G2 | 0.338 | 0.001 |
| B1 - KB | 0.347 | 0.001 |
| B1 - U2 | 0.508 | 0.001 |
| B1 - U7 | 0.47 | 0.001 |
| B1 - U12 | 0.689 | 0.001 |
| B1 - U4 | 0.524 | 0.011 |
| B1 - NTB5 | 0.759 | 0.007 |
| RBN - E | 0.621 | 0.001 |
| RBN - G2 | 0.422 | 0.001 |
| RBN - KB | 0.283 | 0.001 |
| RBN - U2 | 0.498 | 0.001 |
| RBN - U7 | 0.629 | 0.001 |
| RBN - U12 | 0.779 | 0.001 |
| RBN - U4 | 0.737 | 0.005 |
| RBN - NTB5 | 0.911 | 0.001 |
| E - G2 | 0.173 | 0.002 |
| E - KB | 0.382 | 0.001 |
| E - U2 | 0.575 | 0.001 |
| E - U7 | 0.583 | 0.001 |
| E - U12 | 0.917 | 0.001 |
| E - U4 | 0.719 | 0.004 |
| E - NTB5 | 0.912 | 0.001 |
| G2 - KB | 0.065 | 0.032 |
| G2 - U2 | 0.206 | 0.001 |
| G2 - U7 | 0.327 | 0.001 |
| G2 - U12 | 0.598 | 0.001 |
| G2 - U4 | 0.445 | 0.029 |
| G2 - NTB5 | 0.674 | 0.006 |
| KB - U2 | 0.21 | 0.001 |
| KB - U7 | 0.379 | 0.001 |
| KB - U12 | 0.607 | 0.001 |
| KB - U4 | 0.553 | 0.017 |
| KB - NTB5 | 0.691 | 0.003 |
| U2 - U7 | 0.192 | 0.003 |
| U2 - U12 | 0.256 | 0.001 |
| U2 - U4 | 0.316 | 0.085 |
| U2 - NTB5 | 0.41 | 0.041 |
| U7 - U12 | 0.235 | 0.002 |
| U7 - U4 | 0.286 | 0.095 |
| U7 - NTB5 | 0.224 | 0.154 |
| U12 - U4 | 0.783 | 0.004 |
| U12 - NTB5 | 0.775 | 0.002 |
| U4 - NTB5 | 0.037 | 0.500 |

Count data were CLR transformed before analyses with an Aitchison (Euclidean) distance matrix.

**Table S7. ANOSIM results between 4 estuary zones for PICRUSt2 results.**

Tests for differences between unordered Estuary groups

**Global Test**

**Sample statistic (R): 0.406**

**Significance level of sample statistic: 0.001**

Number of permutations: 999 (Random sample from a large number)

**Pairwise Tests**

| Group | R-value | Significance Level |
|---|---|---|
| Lower Estuary - Mid Estuary | 0.345 | 0.001 |
| Lower Estuary - Zinc Factory | 0.544 | 0.001 |
| Lower Estuary - Upper Estuary | 0.585 | 0.001 |
| Mid Estuary - Zinc Factory | 0.348 | 0.001 |
| Mid Estuary - Upper Estuary | 0.465 | 0.001 |
| Zinc Factory - Upper Estuary | 0.149 | 0.001 |

Count data were CLR transformed before analyses with an Aitchison (Euclidean) distance matrix.

**Table S8. Metadata correlations with the inferred functional community composition (CLR transformed).**

| Parameter | PC1 | PC2 | r2 | Pr(>r) |
|---|---|---|---|---|
| d13C | -0.88839 | 0.45909 | 0.4131 | 0.001 |
| Sal_Bot | -0.8408 | 0.54134 | 0.2895 | 0.001 |
| TOC_bot | 0.79775 | -0.60298 | 0.2359 | 0.001 |
| NH4_bot | 0.99158 | 0.12952 | 0.1988 | 0.001 |
| TOC | 0.86873 | -0.49528 | 0.1986 | 0.001 |
| d15N | -0.63787 | 0.77014 | 0.1741 | 0.001 |
| C.N.ratio | 0.90887 | -0.41707 | 0.1641 | 0.001 |
| T_Bot | -0.60457 | -0.79656 | 0.1537 | 0.001 |
| Pb | 0.92966 | -0.36842 | 0.1185 | 0.002 |
| Cu | 0.96896 | -0.24722 | 0.1079 | 0.002 |
| As | 0.99822 | -0.05956 | 0.1032 | 0.003 |
| PO4 | 0.98082 | -0.19492 | 0.0927 | 0.006 |
| TN | 0.76012 | -0.64978 | 0.0841 | 0.008 |
| Hg | 0.91592 | -0.40135 | 0.0703 | 0.02 |
| Zn | 0.99533 | -0.09649 | 0.069 | 0.019 |
| **Fe** | **0.87958** | **-0.47575** | **0.0688** | **0.023** |
| **Cd** | **0.99998** | **-0.00592** | **0.0621** | **0.026** |
| Chla_SFC | 0.78351 | 0.62138 | 0.0192 | 0.337 |
| **O2_bot** | **0.78776** | **0.61598** | **0.0115** | **0.543** |
| PO4_bot | 0.82293 | 0.56815 | 0.0073 | 0.677 |
| **TN_bot** | **0.91642** | **-0.40022** | **0.0027** | **0.895** |
| **Nox_bot** | **0.95166** | **-0.30715** | **0.0019** | **0.911** |

Red data are non-significant parameters.

## References

APHA (2005) 'Standard Methods for the Examination of Water and Wastewater', 21st edn. (American Public Health Association American Water Works Association, and Water Environment Federation: Washington DC, USA)

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