



Supplement of

Carbon amendment stimulates benthic nitrogen cycling during the bioremediation of particulate aquaculture waste

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Table S1. Mean (± standard error) ambient environmental (light, temperature, salinity), nutrient and gas
concentrations recorded in the incubation chambers on day -1 at the start of the light and dark
incubations.

	Light			Dark		
	Mean		SE	Mean		SE
Light (lux)	132.08	±	9.63	-	±	-
Temperature (°C)	29.34	±	0.06	28.62	±	0.04
Salinity (mg L ⁻¹)	35.00	±	0.00	35.00	±	0.00
pH	8.03	±	0.00	8.24	±	0.00
Ammonia (uM)	2.93	±	0.13	2.58	±	0.23
Nitrite (uM)	0.29	±	0.08	0.58	±	0.09
Nitrate (uM)	6.98	±	0.56	7.46	±	0.51
Phosphate (uM)	0.57	±	0.03	0.47	±	0.01
Dissolved inorganic carbon (uM)	2,717.56	±	19.90	2,357.03	±	27.46
Dissolved oxygen (uM)	162.60	±	1.06	166.28	±	1.04
Nitrogen gas (uM)	-	±	-	387.42	±	1.50

- 6 **Table S2.** Overview of the pathways modules and reference profiles within nitrogen metabolism used
- 7 to calculate the predicted relative abundance of genes within each pathway. All data was extracted from
- 8 the Kyoto Encyclopaedia for Genes and Genomes (KEGG) database <u>www.genome.jp/kegg/</u>.

Pathway	Overview	Module	KEGG Ortholog reference profile (KO)
Nitrogen fixation	Nitrogen => ammonia	M00175	K02588 + K02586 + K02591 - K00531
Nitrification	Ammonia => nitrite	M00528	K10944+K10945+K10946 K10535
Denitrification	Nitrate => nitrogen	M00529	(K00370+K00371+K00374+K00373, K02567+K02568) (K00368,K15864) (K04561+K02305,K15877) K00376
Dissimilatory nitrate reduction	Nitrate => ammonia	M00530	(K00370+K00371+K00374+K00373, K02567+K02568) (K00362+K00363,K03385+K15876)
Assimilatory nitrate reduction	Nitrate => ammonia	M00531	(K00367,K10534,K00372-K00360) (K00366,K17877)
Complete nitrification	Ammonia => nitrite => nitrate	M00804	K10944+K10945+K10946 K10535 K00370+K00371

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Fig. S1. Mean (\pm standard error) net fluxes (in µmol m⁻² h⁻¹; n = 5) of: a) dissolved oxygen (DO); b) dissolved inorganic carbon (DIC); c) dinitrogen gas (N₂); and, d) gross primary production (GPP) in incubation chambers under light and dark conditions on day -1, prior to the addition of sea cucumbers and aquaculture waste with (+C) or without (-C) carbon.





Fig. S2. Mean (\pm standard error) benthic light, dark and net fluxes (in µmol m⁻² h⁻¹; n = 5) of: a) phosphate (PO₄³⁻); b) ammonium (NH₄⁺); c) nitrite (NO₂⁻); and d) nitrate and nitrite (NO_x) in incubation chambers under light and dark conditions on day -1, prior to the addition of sea cucumbers and aquaculture waste with (+C) or without (-C) carbon.



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Fig. S3. Nitrogen metabolism pathway map 00910 downloaded from the Kyoto Encyclopaedia 22 for Genes and Genomes (KEGG) database. In the upper part of the diagram, the numbers in the 23 boxes are Enzyme Commission (EC numbers) for enzymes and the chemical reactions they 24 catalyse. In the lower part of the diagram, the enzyme numbers are replaced by the codes for 25 26 the gene that code for each enzyme. Arrows indicate the direction and pathway of the reactions: 27 arrows pointing to the right indicate reduction reactions and arrows pointing to the left indicate 28 oxidation reactions. The circles indicate the different inorganic forms of nitrogen.

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