

Supplementary Material

Title: Metagenomic insights into the structure of microbial communities involved in nitrogen cycling in two integrated multitrophic aquaculture (IMTA) ponds

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Figure S1

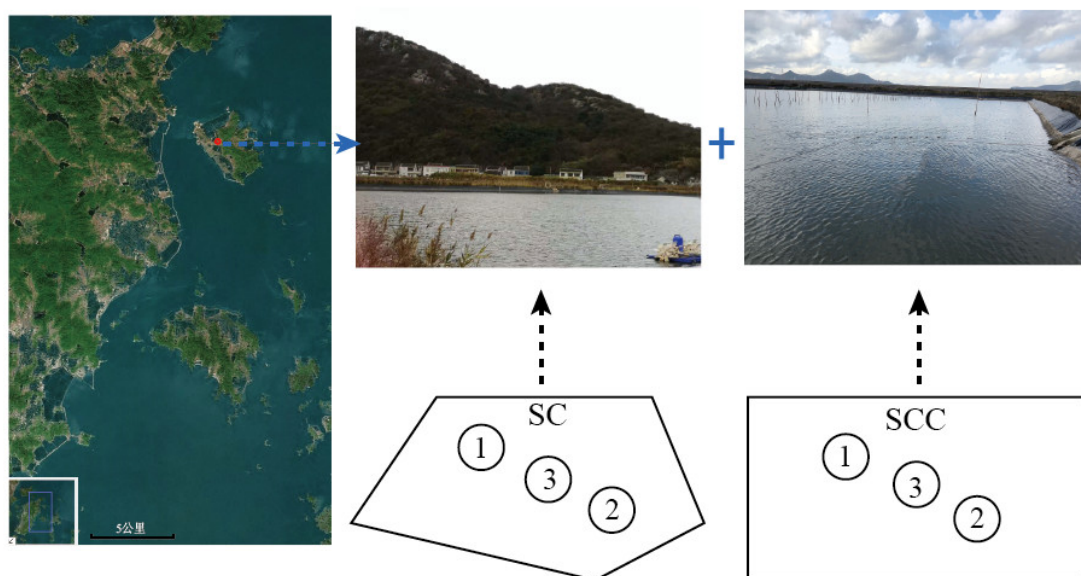


Table S1 Stocking of cultured organisms under SC and SCC.

Treatment	Aquaculture species	Item	Period
SC	<i>Portunus trituberculatus</i>	Carapace length/ cm	0.41±0.02
		Carapace width/ cm	0.74±0.05
		Body weight/ g	0.024
		Density/ kg•ha ⁻¹	7.5
		Body length/ cm	1.02±0.07
	<i>Penaeus japonicus</i>	Body weight/ g	0.0087
		Density/ ind•ha ⁻¹	22.5×10 ⁴
		Carapace length/ cm	0.41±0.02
		Carapace width/ cm	0.74±0.05
		Body weight/ g	0.024
SCC	<i>P. trituberculatus</i>	Density/ kg•ha ⁻¹	7.5
		Body length/ cm	1.02±0.07
		Body weight/ g	0.0087
		Density/ ind•ha ⁻¹	22.5×10 ⁴
		Shell length/ cm	1.85±0.14
	<i>Sinonovacula constricta</i>	Shell width/ cm	0.57±0.10
		Body weight/ g	0.32±0.02
		Density/ kg•ha ⁻¹	37.5

Table S2 PCR parameters.

Reagent	Volume
5×FastPfu Buffer	4 µL
2.5 mmol/L dNTPs	2 µL
Forward Primer (5 µM)	0.8 µL
Reverse Primer (5 µM)	0.8 µL
FastPfu Polymerase	0.4 µL
BSA	0.2 µL
Template DNA	10 ng
ddH ₂ O	20 µL

Table S3 PCR system.

Stage	Reaction
1	1× (3 minutes at 95°C)
2	27×30 sec at 95°C; 30 sec at 55°C; 45 sec at 72°C
3	10 minutes at 72°C, 4°C until halted by user

Table S4 Environmental factors in water from SC and SCC.

Group	Sample sites	T (°C)	pH	DO (mg/L)	Salinity	TN (mg/L)	TP (mg/L)	NH ₄ ⁺ (mg/L)	NO ₂ ⁻ (mg/L)	NO ₃ ⁻ (mg/L)	PO ₄ ³⁻ (ug/L)	Density (kg/ha)
SC	SC1	33.4	9.74	7.732	16.3	3.82	0.35	0.457	0.019	0.863	0.046	0
	SC2	32.6	9.69	10.427	16.6	3.78	0.29	0.477	0.008	1.094	0.031	0
	SC3	31.9	9.72	8.938	16.7	4.38	0.27	0.344	0.010	0.632	0.017	0
SCC	SCC1	34.0	9.94	8.867	16.8	3.18	0.30	0.312	0.015	0.442	0.029	37.5
	SCC2	32.6	9.84	8.583	17.2	3.02	0.22	0.364	0.013	0.531	0.025	37.5
	SCC3	32.7	9.94	8.086	17.1	3.05	0.30	0.381	0.015	0.447	0.024	37.5

Table S5 Environmental factors in sediment from SC and SCC.

Group	Sample sites	T (°C)	pH	ORP (mV)	TC (mg/g)	TN (mg/g)	P_NH ₄ ⁺ (mg/L)	P_NO ₂ ⁻ (mg/L)	C: N	NH ₄ ⁺ (mg/kg)	NO ₂ ⁻ (mg/kg)	Density (kg/ha)
SC	SC1	30.2	7.55	-171	0.1045	0.01015	2.50	0.0005	10.29	4.35	0.0119	0
	SC2	29.8	7.51	-166	0.0709	0.00793	2.05	0.0266	8.94	3.10	0.0360	0
	SC3	30.4	7.54	-183	0.1744	0.00887	1.46	0.0016	19.67	2.63	0.0093	0
SCC	SCC1	29.9	7.62	-167	0.1104	0.00825	4.45	0.0017	13.38	6.05	0.0111	37.5
	SCC2	30.1	7.64	-173	0.0950	0.00852	2.11	0.0017	11.14	3.73	0.0111	37.5
	SCC3	30.3	7.61	-170	0.0958	0.00907	2.17	0.0014	10.57	3.44	0.0108	37.5