Nitrogen enrichment changes the biogeochemical role of sesarmid crabs by shifting their diets in tropical mangrove ecosystems

Xueqin Gao[1,2], Juan Diego Gaitan-Espitia[2], Shing Yip Lee[1,3*]

[1]Simon F.S. Li Marine Science Laboratory, School of Life Sciences, The Chinese University of Hong Kong, Hong Kong SAR, China

[2]The Swire Institute of Marine Science and School of Biological Sciences, The University of Hong Kong, Hong Kong SAR, China

[3] Australian Rivers Institute, Griffith University Gold Coast campus, Southport, Qld 4222, Australia

***Correspondence: joesylee@cuhk.edu.hk**

Abstract

Sesarmid crabs modulate nutrient dynamics of tropical mangroves through their leaf-eating habit. How N enrichment may alter this regulatory role, and the implications for mangrove nutrient dynamics, remain unclear. Using a mesocosm experiment, we tested how N enrichment could change the microphytobenthos (MPB) communities, thus modifying the crab's diet and their role in nutrient dynamics. The factorial experiment combined with field investigation revealed a significant increase in the relative abundance of cyanobacteria. Stable isotope analysis suggested that the main carbon source of crabs shifted from leaf litter to cyanobacteria in mesocosms under both high (20x) and low (2x) N enrichment treatments. The significantly lower total cellulase activity of crabs in the mesocosms might explain the decreased assimilation of carbon from leaf litter. The changes in the MPB and the microbiome with N enrichment in the

presence of crabs may drive significantly higher carbon processing rate in tropical mangroves.

Keywords: Nitrogen, carbon, mangroves, crabs, microphytobenthos, microbiome

Introduction

Anthropogenic nitrogen (N) enrichment has altered N cycling in ecosystems worldwide, leading to ecosystem degradation and decline in the services they provide (Nixon, 1998; Briker et al., 2008; Guignard et al., 2017; Nijssen at al., 2017; Ardón et al., 2021). N enrichment can modify biogeochemical cycles and the trophic state of many ecosystems (Galloway et al., 2008; Gruber and Galloway, 2008; Canfield et al., 2010), altering ecological processes (e.g., bioturbation, nutrient remineralization) in which invertebrates play fundamental regulatory roles (Kremen, 2005; Kristensen, 2008; Lee, 2008; Palumbi et al., 2009; Prather et al., 2013; Nessel et al., 2021). It has been documented, for instance, that N enrichment can drive changes in the invertebrate communities of soft-sediments, reducing macrofaunal functional diversity (Morris and Keough, 2003; Fitch and Crowe, 2012; Posey et al., 2006; Douglas et al., 2017). This might affect the resilience of ecosystems to environmental changes due to decreased ecosystem complexity and trophic interactions (Hulot et al., 2000; Douglas et al., 2017). This patten is evident in many coastal and aquatic ecosystems, where nutrient enrichment has become a pervasive threat to invertebrate communities (Nessel et al., 2021). However, not all nutrients are equally important for these communities. Changes in the availability of limited nutrients, for instance, often favour some species over

44 others, causing biodiversity loss (Isbell et al., 2013), potentially reducing the ability of
45 ecosystems to withstand future stresses. Such negative impact on species diversity also
46 exacerbates other ecological problems leading to declines in ecosystem productivity
47 (Villnäs et al., 2013; Isbell et al., 2013).

48 Mangrove forests are often distributed in estuaries, which are at the receiving end of
49 nutrient-enriched riverine waters resulting from the discharge of domestic and
50 agricultural wastes (Lee, 2016). These ecosystems can provide many ecological
51 services, including improving water quality (MacDonnell et al., 2017), and
52 biogeochemical regulation though the absorption of nutrients via mangrove soils
53 (Cannicci et al., 2009; Tam et al., 2009; Ouyang and Guo, 2016). However, an
54 increasing number of studies indicate that chronic nutrient enrichment may negatively
55 affect mangrove ecosystem and the associated benthos. Previous field (Wear and
56 Tanner, 2007; Capdeville et al., 2018, 2019; Theuerkauff et al., 2018, 2020) and
57 mesocosm (Penha-Lopes et al., 2009) studies have reported modification of the
58 distribution of macrofauna and a decrease in population or species richness in
59 mangroves subjected to excess nutrient inputs. Such biodiversity decline could be
60 associated to the greater physiological sensitivities and functional constraints of some
61 species to enhanced nutrient loads in mangrove ecosystems. For instance, crabs have
62 an integrated metabolism that shows negative impacts induced by wastewater, with a
63 burst in oxygen consumption that may be caused by osmo-respiratory trade-offs arising
64 from compromised gill functioning (Theuerkauff et al., 2018, 2020). These

physiological responses could partially explain the documented decrease in the abundance of crabs exposed to wastewater (Mégevand et al., 2021).

Extensive research has found that nutrient enrichment leads to regime shifts in the dominant producers of aquatic ecosystems, from macrophytes to macro/microalgae (Scheffer et al., 2001). Blooms of these organisms can be toxic and/or lethal (e.g., through hypoxia) to many aquatic animals (Paerl et al., 2004; Diaz and Rosenberg, 2008). The classical bottom-up model of faunal assemblages regulated by nutrient supply predicted that nutrient enrichment could increase the growth of benthic microalgae and bacteria in sediment, increasing the populations of grazers (McQueen et al., 1989). However, top-down effects such as grazing may constrain the impact of nutrient enrichment, e.g., grazers suppressed a bottom-up response of algae after two years of nutrient enrichment in an Alaskan river (Peterson et al., 1993). Assessing the relative responses of both bottom-up and top-down effects to nutrient enrichment is essential to understand the consequences of eutrophication at the whole ecosystem level.

In mangroves, in addition to the macrophytes, the microphytobenthos (MPB) also contribute to the overall primary production and play an important role at the base of the benthic food web (Hope et al., 2020a). The ecological dynamics of these microorganisms (i.e., community structure and productivity) is tightly regulated by nutrient availability in mangrove ecosystems(Benny et al., 2021). Nutrient-driven changes in the relative proportion of different microalgal groups ultimately affect the quantity and quality of food available to benthic consumers (Hope et al., 2020b). From

these groups, sesarmid crabs are often the dominant invertebrate group in tropical mangroves, playing an essential role in ecosystem processes and functioning due to their leaf-eating habits (Lee, 2008). The trophic ecology of these important consumers is highly dependent of MPB as one of the main N sources (Gao and Lee, 2022). Our understanding of the general responses of mangrove ecosystems and their biotic components (MPB, fauna and flora), especially the sesarmid crabs, to changes in nutrient inputs is still limited. There is a need to better understand how nutrient enrichment may affect fundamental ecological and functional activities (e.g., feeding) of sesarmid crabs and thus their role in mangrove biogeochemical processes.

In this study, a mesocosm experiment was performed to investigate how sesarmid crabs responded to nutrient enrichment and their regulatory role in nutrient dynamics under eutrophic conditions. The feeding rate and digestive enzyme activities of invertebrates had been shown to be sensitive to nutrient enrichment (Boldina-Cosqueric et al., 2010; Charron et al., 2015; Dedourge-Geffard et al., 2013; Mégevand et al., 2021). Organisms exposed to pollutants may have to increase their energy requirements to maintain basal metabolism, to the detriment of growth and reproduction (Calow and Sibly, 1990; De Coen and Janssen, 1998; Koehn and Bayne, 1989). Species-specific dietary characteristics such as feeding habits and dominant food sources might be important in determining the population- and community-level implications of consumer responses to nutrient enrichment in aquatic ecosystems (Cross et al., 2005). We hypothesized that exposure to N enrichment would change the abundance and

species composition of the MPB in surface sediment. Such change would then affect the diets of sesarmid crabs, e.g., consuming more MPB and processing less leaf litter, and thus their role in biogeochemical cycles. By directly linking nutrient dynamics to the genetic composition of microbial communities, this study provides mechanistic insights into the biogeochemical processes driven by sesarmid crabs under eutrophic conditions.

Materials and methods

General approach

We used a mesocosm experiment employing ^{13}C-labelled mangrove leaves in the presence of other food sources in the sediment (i.e., MPB). Through this, we aimed to investigate how sesarmid crabs responded to ammoniacal N (^{15}N-labelled) enrichment in tidal water and the regulatory role of these animals in nutrient dynamics. For two months, isotopically enriched leaf litter was added to mesocosms inundated by tidal water with high or low N enrichment in the presence/absence of crabs. We then compared MPB abundance and composition in the surface sediment of the mesocosms with different treatments, combining with stable isotope analysis and assessment of cellulase enzyme activity to see how N enrichment might affect the diet of sesarmid crabs. To compare with our mesocosm experiment, we assessed the MPB composition in the surface sediment and gut content of sesarmid crabs as well as, the cellulase activity of these animals in the field. Moreover, we compared the ^{13}C and ^{15}N enrichment levels of each trophic compartment (i.e., sediment, porewater, MPB,

mangrove seedlings, crabs) to assess the influence of the crabs on C and N dynamics within the mesocosms. Finally, we compared the biomass of mangrove seedlings to assess the extent to which C/N changes might affect the primary productivity of mangrove ecosystems.

Sample collection

The sesarmid crab *Parasesarma bidens*, a common species associated with mangrove forests in East Asia, was used in the experiment. Male individuals of *P. bidens* (carapace width from 11 to 20 mm) and sediment samples were collected from the *Kandelia obovata* mangrove forest at Ting Kok (22°28'23"N,114°12'50"E), Hong Kong. Crabs were starved for two days before transferring to the mesocosms. Mangrove propagules collected three months in advance were planted individually in plastic containers for isotopic labelling and subsequent use in the mesocosm experiment. Forty-eight individual seedlings each with two leaves were randomly transferred into the 12 experimental mesocosms, and the remaining seedlings were used for the preparation of ^{13}C-enriched mangrove leaves.

Preparation of ^{13}C-enriched mangrove leaves

The ^{13}C enrichment started when propagules grew to seedlings with four to six leaves each. The labelling followed Putz et al. (2011) with some modification. ^{13}C-urea solution was brushed onto the upper and lower surfaces of the mangrove leaves using a small paintbrush. ^{13}C-urea solution was prepared by dissolving 100 mg 99-atom% ^{13}C-urea in 50 ml MilliQ water. To ensure good contact with the leaf surface, 12.5µl of

wetting agent was added to the solution. ^{13}C labelling was applied once a day for 15 consecutive days. The ^{13}C enrichment levels of the mangrove leaves were measured before the mesocosm experiment.

Mesocosm experiment

Twelve mesocosms were set up in an outdoor area at the marine lab of The Chinese University of Hong Kong. Each mesocosm setup included two tanks (68cmx58cmx40cm); the upper tank with one water inlet and two water outlets (setting the maximum and minimum water levels, respectively) simulating the tidal mangrove forest and the lower tank acted as a water reservoir with a submersible pump and a timer switch programmed to simulate the semi-diurnal tidal variations. The upper tanks were filled with mangrove sediment collected from Ting Kok to a depth of 10 cm. Four *K. obovata* seedlings were planted in each tank (48 in total). Tidal water was pumped into the upper tank from the lower reservoir during high tide. The timer was programmed to provide inundation of the substrate for 1.5 hours during each flood tide (12 hours). There were four treatments in total, Treatment 1 and 2 having low N enrichment (0.1 mg L^{-1} N - $^{15}NH_4Cl$) with and without crabs, respectively. Treatment 3 and 4 had high N enrichment (1 mg L^{-1} N - $^{15}NH_4Cl$) with and without crabs, respectively. There were three replicate mesocosms for each treatment. The tidal water for each mesocosm was supplied as 100 L of seawater with a salinity at 18 to 20 and background ammonium-N at 0.05 mg L^{-1}. Three male individuals of *P. bidens* were kept in each of the "with crabs" mesocosms.

The mesocosm experiment started in May 2022 during the active season of crabs. The same amount (according to the litterfall rate in the field: 1.58 g m^{-2} d^{-1}; Lee, 1990) of ^{13}C-enriched mangrove leaves with the same enrichment level was added to all mesocosms (the four treatments). The leaf ingestion rate was assessed by measuring the area of the leaves before and after introduction to the mesocosms for 24 hours. After one month, crabs were collected from the mesocosms (both low and high N enrichment treatments) for stable isotope analysis and assessment of cellulase activity. The moulting frequency of crabs was also recorded during the experimental period. The top 5 mm surface sediment was collected from all the mesocosms for analysis of MPB composition and abundance as this layer supported most of the MPB due to limited light penetration (Jesus et al., 2006). Then new crabs were added to the "with crabs" mesocosms at the same density. After another month, all the crabs, mangrove seedlings, and samples of the surface sediment, MPB, and porewater were collected from the mesocosms, and prepared for stable isotope analysis and the measurement of selected variables relevant to productivity (seedling growth, MPB abundance; Table 1). Porewater was collected for $\delta^{13}C$ analysis of dissolved inorganic carbon (DIC). MPB was extracted from the top 5 mm surface sediment using centrifugation in LUDOX™ (see below). Surface sediment (top 5 mm) was also collected for C and N content measurement and analysis of the microbial communities.

Table 1 Sampling during the mesocosm experiment for analysis of key environmental variables

Samples	Processing	Stable isotope analysis (SIA)	Other variables
Crabs	Muscle tissues taken from their claw for SIA Hepatopancreas taken for cellulase activity analysis	$\delta^{13}C$ and $\delta^{15}N$	Moulting rate Cellulase activity
Seedlings	Separate leaves and roots, washed with MilliQ water, dried at 60 ℃ for 2 days, n=4 for each mesocosm	$\delta^{13}C$ and $\delta^{15}N$	Biomass and C, N content (%) of different parts
Surface sediment	Top 5 mm sediment, n=3 for each mesocosm, dried at 60 ℃ for 2 days	$\delta^{13}C$ and $\delta^{15}N$	C, N content (%)
Microphytobenthos (MPB)	Top 5 mm sediment, extraction using LUDOX, n=3 for each mesocosm	$\delta^{13}C$ and $\delta^{15}N$	Species composition Abundance
DIC in porewater	Placed 20 ml porewater in 30-ml air-tight vial, injected 1ml HCl solution (0.1N) with syringes, collected CO_2 produced.	$\delta^{13}C$	
Microbial communities	Top 5 mm sediment, DNA extraction, 16s rRNA sequencing		Species composition Relative abundance

191

192

193 **Assessment of MPB abundance and composition**

194 MPB abundance in surface sediment was estimated spectrophotometrically by
195 quantifying the concentration of chlorophyll-a using the aqueous acetone extraction
196 method, with three replicates for each mesocosm and nine replicates of the field
197 samples (Aminot and Rey, 2000). MPB composition in the surface sediment and gut
198 contents of the crabs was estimated by counting the frequency of cyanobacteria and
199 diatoms in sediment samples of the same mass using a light microscope (200x) over a

grid, with three replicates for each mesocosm and fifteen replicates of the field samples. The species richness of the MPB samples were recorded at the same time.

MPB extraction

MPB was isolated from the sediment by centrifugation in colloidal silica (LUDOX^{TM}) (Bui and Lee 2014; Gao and Lee 2022). The sediment sample was washed through a series of decreasing mesh sizes: 2-mm, 500µm, 250µm, 45µm and 5µm. The material retained on the 5µm sieve was then transferred to 50ml falcon tubes. After settling at 4°C overnight, the clear supernatant was removed carefully while avoiding disturbance to the sediment at the bottom. 23ml of colloidal silica solution (1.340 g ml^{-1} density) was then added to each tube containing the sample, mixed and centrifuged (4000 rpm at 4°C) for 10 mins. The entire top green layer (MPB) was isolated and washed with MilliQ water, then transferred into a tin capsule, dried and weighed for stable isotope analysis.

Assessment of total cellulase activity

Crabs were anaesthetized and euthanized on ice for sampling of the hepatopancreas, which is the main organ of crustaceans involved in producing cellulases (Adachi et al., 2012). Thus, quantification of cellulase activity in the hepatopancreas allows an assessment of how environmental changes such as nutrient enrichment may affect the feeding activities of sesarmid crabs. All samples of hepatopancreas were diluted in 200 µl Milli-Q water and homogenized using a stainless-steel tool. The homogenate was centrifuged for 12 min at 15,000 rpm and 4°C to eliminate lipids and other tissue debris, and the supernatant containing digestive enzyme proteins was collected and stored in

aliquots at -80°C. Total cellulase activities in the hepatopancreas were determined by measuring the glucose concentration produced from microcrystalline cellulose (Sigma) following the methods of Bui and Lee (2014) with some modifications. Each reaction was set up by mixing 20 µl of hepatopancreas juice with 200 µl of 2% microcrystalline cellulose, incubated in an orbital shaking incubator at 150 rpm. After incubation for two hours, the assay tubes were transferred to an ice slurry to terminate the reaction immediately. The amount of glucose produced in the reaction was quantified by the D-Glucose Hexokinase assay kit (Megazyme) according to the manufacturer's instructions. All assays were conducted in triplicate. The amount of glucose measured in each sample was corrected with the amounts of glucose present initially in the respective hepatopancreas juice samples. The total cellulase activity was defined as the amount of glucose produced in each assay per hour per ml of hepatopancreas juice.

Field investigation

Field investigation was conducted in the same mangrove forest (Ting Kok) where the materials (crabs, sediment, and propagules) for the mesocosm experiment were collected. A total of 15 male *P. bidens* were collected from 15 sites (distance between each site >1 m) of the mangrove forest for gut content, stable isotope, and cellulase activity analysis. Meanwhile, surface sediment was collected from the same site where each crab was collected for analysis of MPB abundance (Chla) and composition (n=15). Cyanobacteria mats were also collected from the same site for stable isotope analysis

(n=5). All the crabs and sediment samples were kept cool immediately after collection before being taken to the laboratory for analysis.

Stable isotope analysis and the relative contributions of food sources

The C and N content as well as stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of the surface sediment, mangrove seedlings (leaves and roots), crab tissues (muscle), and MPB samples were measured with a Thermo Analytical elemental analyser, Flash EA 1112 Series coupled via a ConFlo IV interface to a Thermo Delta V Plus isotope ratio mass spectrometer. $\delta^{13}C$ of DIC in porewater was measured with a Picarro G2201-i spectrometer. Stable isotope ratios are expressed in δ notation (per mil, ‰) relative to the conventional standards (Vienna Pee Dee Belemnite for C and atmospheric N_2 for N) according to:

$$\delta X\,(‰) = [(R_{sample}/R_{standard}) - 1] \times 1000$$

where $X = {}^{13}C$ or ${}^{15}N$, and $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$. Measurement precision was better than 0.3‰ for both $\delta^{13}C$ and $\delta^{15}N$. The contribution of mangrove leaves and MPB to the C and N in the tissues of *P. bidens* in the mesocosms and in the field were estimated by the mixing model MixSIAR (Moore and Semmens, 2008).

16S rRNA gene sequencing and bioinformatics analysis

Total genomic DNA of the surface sediment of each mesocosm was extracted using a DNeasy PowerSoil Kit (QIAGEN, USA) following the manufacturer's protocol. DNA yield and quality were checked using a NanoDrop 1000 spectrophotometer

(Thermo Fisher Scientific, Wilmington, DE, USA). The V4 region of the 16S rRNA gene was amplified following a one-step PCR protocol (Kozich et al., 2013) with the following cycle conditions: 95 ◦C for 5 min; followed by 28 cycles of 95 ◦C for 20 s, 55 ◦C for 15 s, 72 ◦C for 30 s; followed by 5 min extension at 72 ◦C. All PCR products are purified by Agencourt AMPure XP beads, dissolved in Elution Buffer and eventually labelled to finish library construction. Library size and concentration were detected by Agilent 2100 Bioanalyzer. Qualified libraries were sequenced on HiSeq platform according to their insert size. The OTUs were classified using a 97% identity threshold. Taxonomic classifications were assigned to OTU representative sequence using Ribosomal Database Project database (RDP) at a minimum confidence level of 80% (Cole et al., 2014). Taxonomic analysis of OTU representative sequences was carried by RDP classifier Bayesian algorithm to identify the composition of microbial structure. The diversity characteristics (Shannon, Simpson, Chao, Ace, and coverage) of the microbiome were determined using PERMANOVA, while the partial least squares discrimination analysis (PLS-DA) (Barker and Rayens, 2003) was conducted to compare the difference between groups using R software package “mixOmics”. To find more detailed differences in the bacterial compositions in surface sediment of the mesocosms among the four treatments, a LEfSe analysis was performed using the online version of Galaxy (Segata et al., 2011).

Statistical analyses

Two-way ANOVAs were performed to test the effects of the presence/absence of crabs and N enrichment on MPB composition and abundance. One-way ANOVA followed by post hoc Tukey's tests were performed to test differences in cellulase activity of sesarmid crabs among different groups (in the field, in mesocosms with high or low N enrichment). The difference in stable isotope enrichment level (indicated by stable isotopic values) of sesarmid crabs was compared between the two enrichment treatments (high or low N enrichment) using two-sample t-tests. All analyses were performed at α=0.05 using SPSS 28.0.

Results

MPB composition, abundance, and species richness

The proportions of cyanobacteria and diatoms showed significant differences in their average contributions to the MPB among the four treatments (Table 2). In the presence of crabs, the average cyanobacteria contribution to total MPB in surface sediment was lower than in the absence of these animals (With crabs: 40.4% and 64.4%; Without crabs: 86.2% and 92.9% for low and high N enrichment, respectively) (Table 2). The average cyanobacteria contribution in the field (surface sediment in mangrove forests) was 7.74%, which was much lower compared with those in the mesocosms. The average cyanobacteria contribution

in the gut content of sesarmid crabs (*P. bidens*) in the field was 56.8%, which was about seven-fold higher than that in the surface sediment where the animals were collected

(Table 2). The results of a two-way ANOVA showed that both, N enrichment and crab presence/absence, had significant effects on MPB composition (Table 3).

Table 2 MPB composition (mean ± SD percentages of cyanobacteria and diatom in total MPB) and MPB species richness of surface sediment (top 5 mm) and gut content of crabs (*P. bidens*) in the mesocosms and in the field (Ting Kok mangrove forest).

	Treatment	% cyanobacteria	% diatom	Number of MPB species	MPB abundance (Chla: µg g^{-1})
Mesocosm surface sediment	Low N enrichment without crabs	86.2±5.7	13.8±5.7	7	16.7±3.1
	Low N enrichment with crabs	40.4±6.9	59.6±6.9	13	18.9±2.9
	High N enrichment without crabs	92.9±10.8	7.1±10.8	5	15.7±4.6
	High N enrichment with crabs	64.4±4.2	35.6±4.2	10	13.9±2.8
Field	Surface sediment	7.7±10.4	92.3±10.4	22	11.9±1.3
	Gut content of crabs	56.8±22.8	43.2±22.8		

MPB species richness was lower (between 5 to 13 species) in the mesocosms compared to the field samples (22 species). In the former, the presence of crabs was associated to higher MPB species richness, particularly under low N conditions (13 species). The absence of crabs as associated to low species richness (5 species), particularly under the high N treatment (5 species) (Table 2). MPB abundance (Chla concentration) in both low and high N enrichment mesocosms was significantly higher than that in the field ($p<0.05$) (Table 2). From these two factors assessed, only N

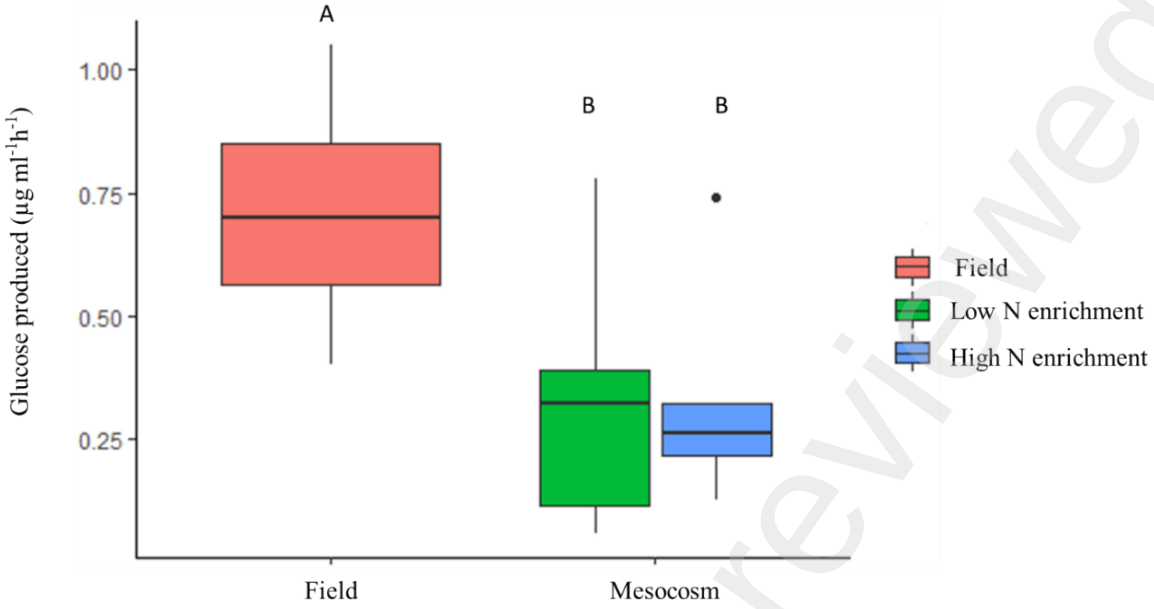
enrichment had a significant effect on MPB abundance (Factorial ANOVA; $p<0.05$) in the mesocosms (Table 3).

Table 3 Results of a two-way ANOVA on the effects of nitrogen enrichment and crabs on MPB composition and abundance in the surface sediment. Significant differences ($p < 0.05$) are indicated in **bold**.

Dependent variable: MPB composition (% Cyanobacteria)				
Sources	df	Mean Square	F	p value
Nitrogen	1	1.240	229.72	**<0.001**
Crab	1	0.213	39.45	**<0.001**
Nitrogen * Crab	1	0.068	12.61	**0.001**
Error	8	0.674		
Dependent variable: MPB abundance (Chla)				
Sources	df	Mean Square	F	p value
Nitrogen	1	38.03	6.685	**0.014**
Crab	1	78.42	0.055	0.817
Nitrogen * Crab	1	0.642	2.986	0.094
Error	32	0.896		

Cellulase activity in the hepatopancreas of crabs

The total cellulase activity in the hepatopancreas of crabs in the mesocosms was significantly lower than that of their counterparts in the field ($p<0.05$). There was no significant difference between the two N enrichment treatments of mesocosms (Figure 1).



329

330 Figure 1. Total cellulase activity (glucose produced) of sesarmid crabs in the field and
331 mesocosms. Values with different letters are significantly different ($p<0.05$).

332 **Stable isotope values and contribution ratio of food sources**

333 After one month, the crabs became enriched in ^{13}C and ^{15}N in both high and low N
334 enrichment mesocosms compared to the field crabs (Table 4). Moulting rate of the crabs
335 during the experiment was higher in the high N enrichment mesocosms (55.6%) than
336 that in low N enrichment mesocosms (22.2%), but leaf consumption rate followed the
337 opposite trend. There was no significant difference in $\delta^{13}C$ of the crabs between the
338 high and low N enrichment treatments. Both $\delta^{13}C$ and $\delta^{15}N$ values of the crabs were
339 closer to those of the cyanobacteria in their respective mesocosms than those of the
340 leaves added to the mesocosms. In the low N enrichment mesocosms, the contribution
341 of mangrove leaves to C and N in crabs were 19.4% and 29.5%, respectively, with MPB
342 (cyanobacteria mats) contributions at 80.6% and 70.5%, respectively. In the high N

343 enrichment mesocosms, the corresponding values for mangrove leaves were 17.7% and
344 28.7%, respectively, while those from MPB (cyanobacteria mats) were 82.3% and
345 71.3%, respectively (Table 4).

346 Table 4 Stable isotope values of crabs and their food sources, and the contribution of
347 food sources to C and N in crabs based on mixing models in the mesocosms at the end
348 of the experiment.

		Field	Mesocosms	
			High N enrichment	Low N enrichment
Ingestion rate of leaves by crabs		57% Lee (1989)	54.8%	63.5%
Moulting rate during the experiment			55.6% (5/9)	22.2% (2/9)
Isotopic value of leaves (average ± SD)	$\delta^{13}C$	-28.9±0.5‰	177±42‰	177±42‰
	$\delta^{15}N$	6.8±0.6‰	9.5±1.0‰	9.5±1.0‰
Isotopic value of cyanobacteria mats (MPB) (average ± SD)	$\delta^{13}C$	-17.2±0.3‰	-13.8±1.9‰	-15.3±2.2‰
	$\delta^{15}N$	9.7±0.2‰	5369±311‰	382±63‰
Isotopic value of crabs (average ± SD)	$\delta^{13}C$	-24.5±1.2‰	-4.5±8.4‰	-4.8±6.0‰
	$\delta^{15}N$	9.2±0.7‰	3832±219‰	272±54‰
Contribution of leaves to crabs	C	62.5%	17.7%	19.4%
	N	17.3%	28.7%	29.5%
Contribution of MPB to crabs	C	37.5%	82.3%	80.6%
	N	82.7%	71.3%	70.5%

349 **Enrichment level of each compartment of the mesocosms**

350 After two months, sediment, porewater DIC and MPB in mesocosms with crabs were
351 significantly more enriched in ^{13}C than those without crabs for both high N and low N
352 enrichment (Figure 2). Both main factors, N enrichment (high/low) and crabs
353 (presence/absence) had a significant effect on the ^{13}C enrichment level of porewater

DIC and MPB. However, only the presence/absence of crabs had a significant effect on the ^{13}C enrichment level of sediment in the mesocosms (Table 5). Leaves and roots of the seedlings showed no significant difference in $\delta^{13}C$ among the four treatments and showed no significant differences with seedlings in the field. The sediment, MPB and plants (leaves and roots) in the mesocosms with high N enrichment were about 10-fold enriched in ^{15}N compared with those in the mesocosms with low N enrichment. However, there was no difference in ^{15}N between mesocosms with and without crabs for both enrichment treatments (Figure 3).

C, N content and productivity

Higher C and N contents of surface sediment were observed in mesocosms with crabs for both high and low N enrichment (Figure 4 and Figure 5). Contrary to N, the crab presence/absence had a significant effect on C and N content ($p<0.05$) in the mesocosms (Table 5). For high N enrichment mesocosms the C and N content was 17.9% and 26.6% higher in mesocosms with crabs than those without crabs, respectively. For low N enrichment mesocosms the C and N content was 20.3% and 25.2% higher in mesocosms with crabs than those without crabs, respectively. The N content of leaves from mangrove seedling was higher (25%) in mesocosms with high N enrichment (1.95±0.31) (average ± SD) compared to the low N enrichment (1.55±0.20). The belowground and total biomass of mangrove seedlings in high N enrichment mesocosms with crabs was significantly higher (14.4% and 18.1% respectively) than

374 those without crabs ($p<0.05$). aNo difference in belowground or total biomass was
375 found in mesocosms with low N enrichment (Figure 6).

376 Table 5 Results of a two-way ANOVA on the effects of N and crab treatment on the
377 level of ^{13}C- enrichment of each compartment and C and N content of surface sediment
378 of the mesocosms. Significant differences ($p < 0.05$) are indicated in **bold**.

Dependent variable: ^{13}C-enrichment level of sediment				
Sources	df	Mean Square	F	p value
Nitrogen	1	5.97	0.96	0.334
Crab	1	506.69	81.44	**<0.001**
Nitrogen x Crab	1	0.047	0.008	0.931
Error	32	6.221		
Dependent variable: ^{13}C-enrichment level of DIC				
Sources	df	Mean Square	F	p value
Nitrogen	1	13.85	17.55	**0.003**
Crab	1	101.55	128.75	**<0.001**
Nitrogen x Crab	1	3.51	4.45	0.068
Error	8	0.789		
Dependent variable: ^{13}C-enrichment level of MPB				
Sources	df	Mean Square	F	p value
Nitrogen	1	20.74	30.61	**0.001**
Crab	1	42.57	62.81	**<0.001**
Nitrogen * Crab	1	1.80	2.66	0.142
Error	8	0.678		
Dependent variable: C content of sediment				
Sources	df	Mean Square	F	p value
Nitrogen	1	0.49	1.89	0.179
Crab	1	3.36	12.9	**0.001**
Nitrogen * Crab	1	0.15	0.58	0.812
Error	32	0.260		
Dependent variable: N content of sediment				
Sources	df	Mean Square	F	p value
Nitrogen	1	0.06	3.06	0.09
Crab	1	0.15	8.01	**0.008**
Nitrogen * Crab	1	0.01	0.07	0.799
Error	32	0.002		

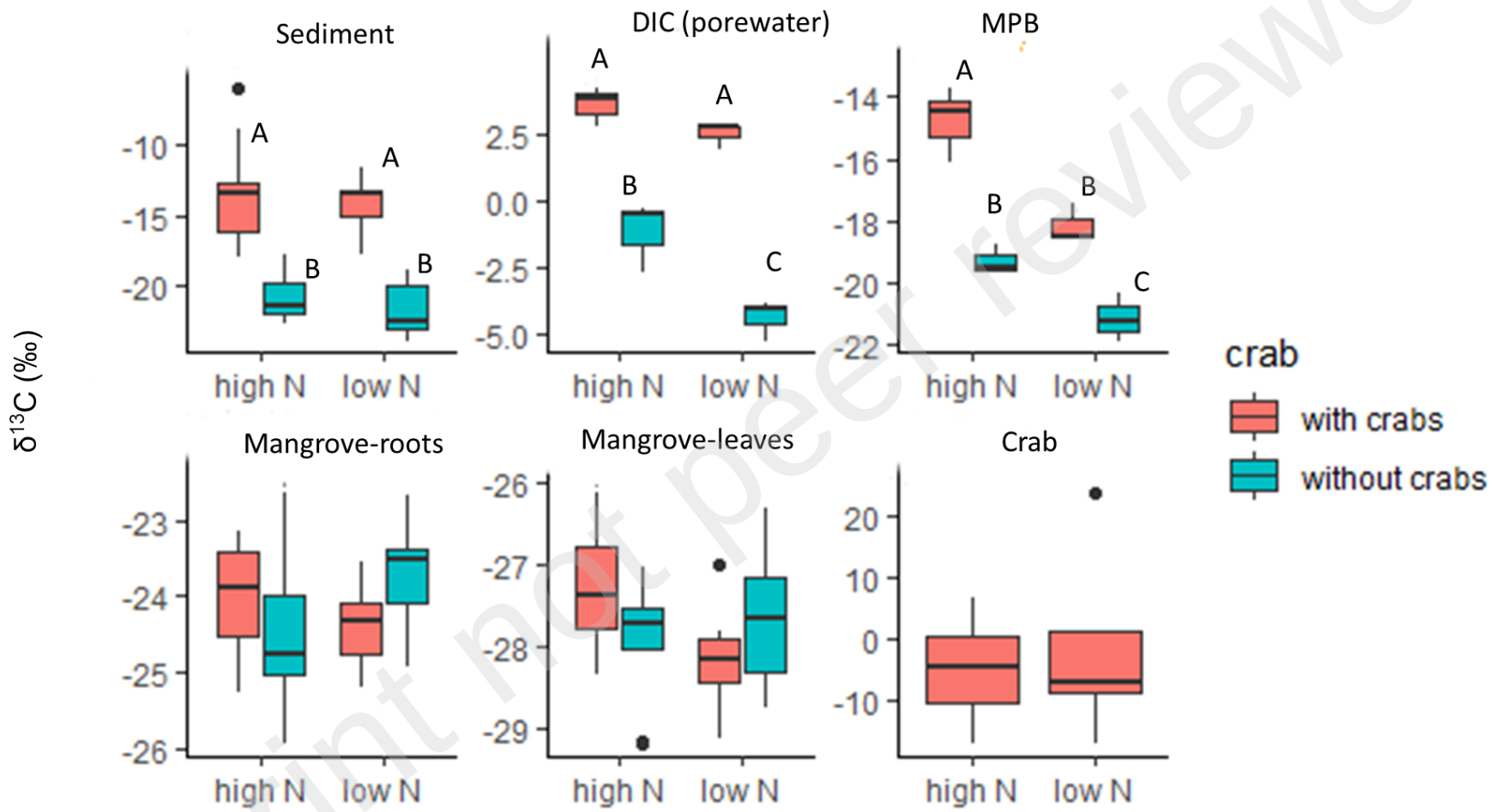
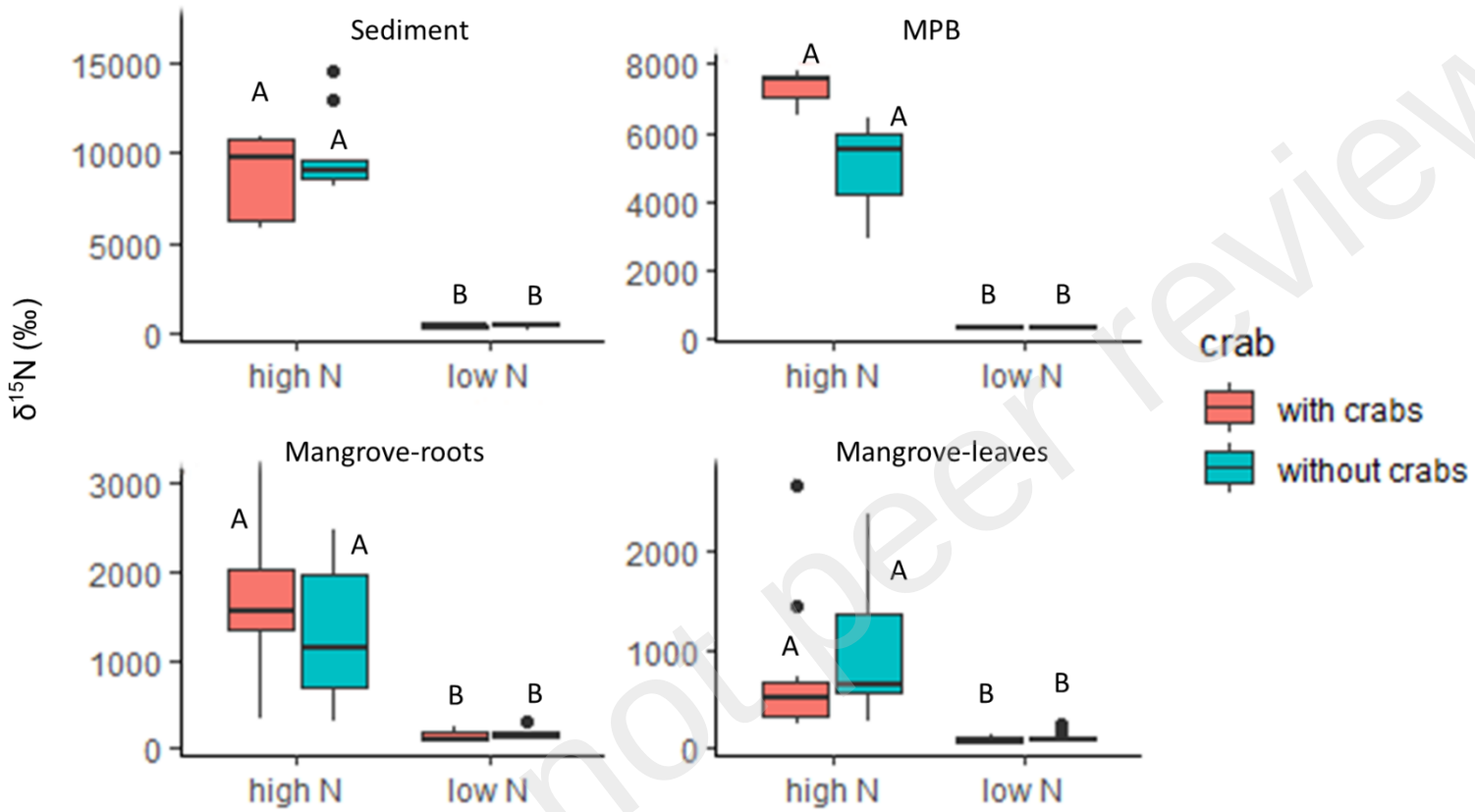


Figure 2. ^{13}C-enrichment level of each compartment of the mesocosms with high and low N enrichment. Values with different letters are significantly different ($p<0.05$).

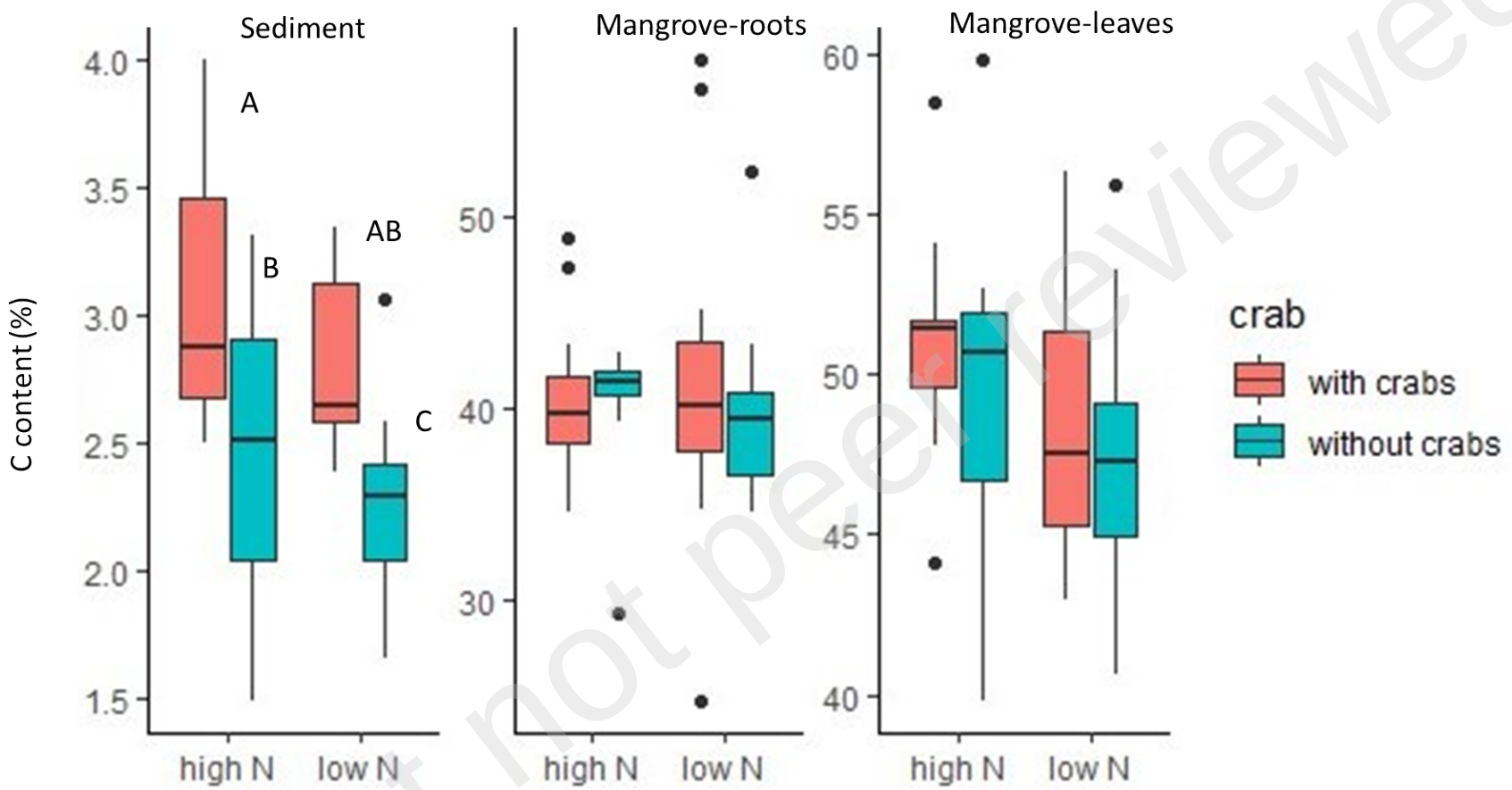


384

385 Figure 3. ^{15}N-enrichment level of each compartment of the mesocosms with high and low N enrichment. Values with different letters
386 are significantly different ($p<0.05$).

387

388



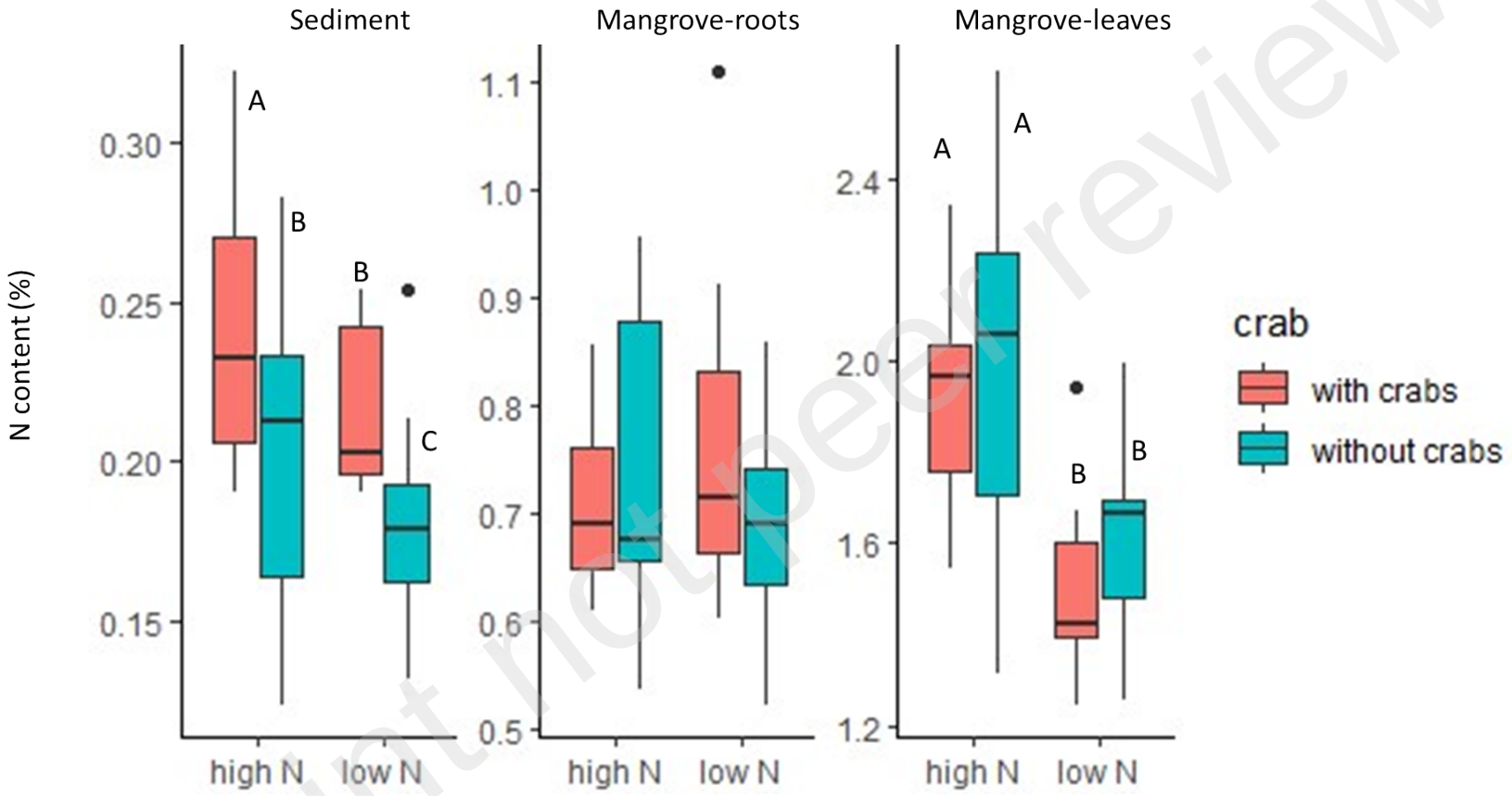
389

390 Figure 4. C content (%) of surface sediment and mangrove seedlings (roots and leaves) in the mesocosms with high and low N

391 enrichment. Values with different letters are significantly different ($p<0.05$).

392

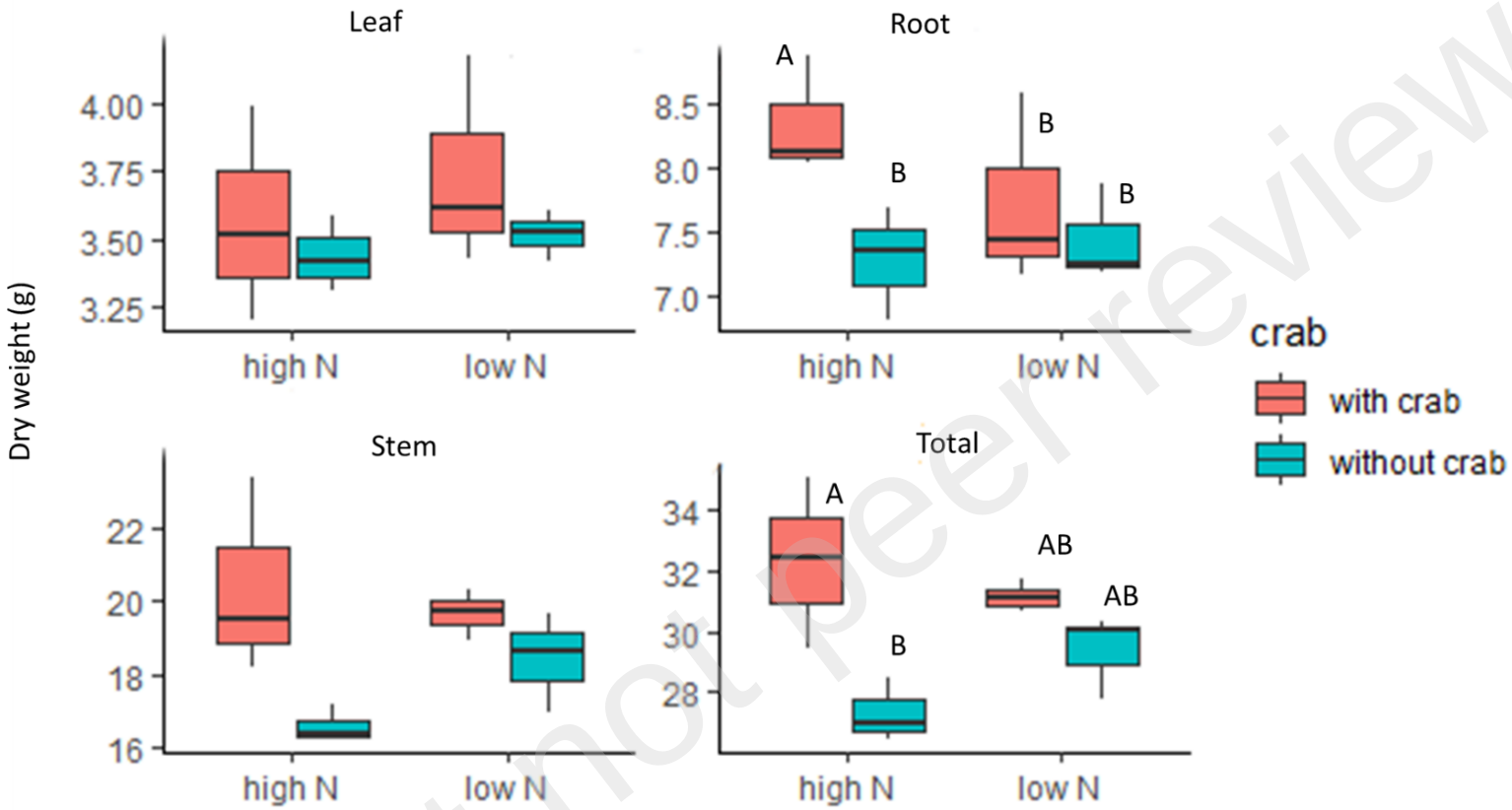
393



394

395 Figure 5. N content (%) of surface sediment and mangrove seedlings (roots and leaves) in the mesocosms with high and low N
396 enrichment. Values with different letters are significantly different ($p<0.05$).

397



398

399 Figure 6. Biomass of the seedlings in the mesocosms with high and low N enrichment (total = leaf + root + stem). Values with
400 different letters are significantly different ($p<0.05$).

Microbial communities in surface sediment

Bacterial community structures in surface sediment of mesocosms with different treatments were analysed and compared based on 16S rRNA amplicon sequencing. The total number of observed bacterial species and alpha diversity (Chao, Ace, Simpson) showed no significant difference among the four treatments. However, significant differences were observed for the Shannon index and Good's coverage ($p<0.05$). Shannon index showed that the highest bacterial biodiversity occurred in the high N enrichment mesocosms with crabs and the lowest occurred in low N enrichment mesocosms without crabs (Figure 7). The partial least squares discrimination analysis (PLS-DA) of OUT separated the four treatments into 3 different groups: (1) high N enrichment with crabs; (2) low N enrichment with crabs; and (3) high and low N enrichment without crabs (Figure 8).

Linear discriminant analysis Effect Size (LEfSe) showed that mesocosms of low N enrichment with crabs were more highly colonized by Actinobacteria. The high N enrichment sediments with crabs were more highly colonized by Flavobacteriales, whereas in mesocosms without crabs (both high and low N enrichment), no special families or orders were found (Figure 9). For the relative abundance of the top 10 bacterial classes, we found f significant differences between high and low N enrichment mesocosms with crabs for, Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Flavobacteriia and Sphingobacteriia. Only one class (Cytophagia) showed significant differences between high and low N enrichment mesocosms without crabs (Figure 10).

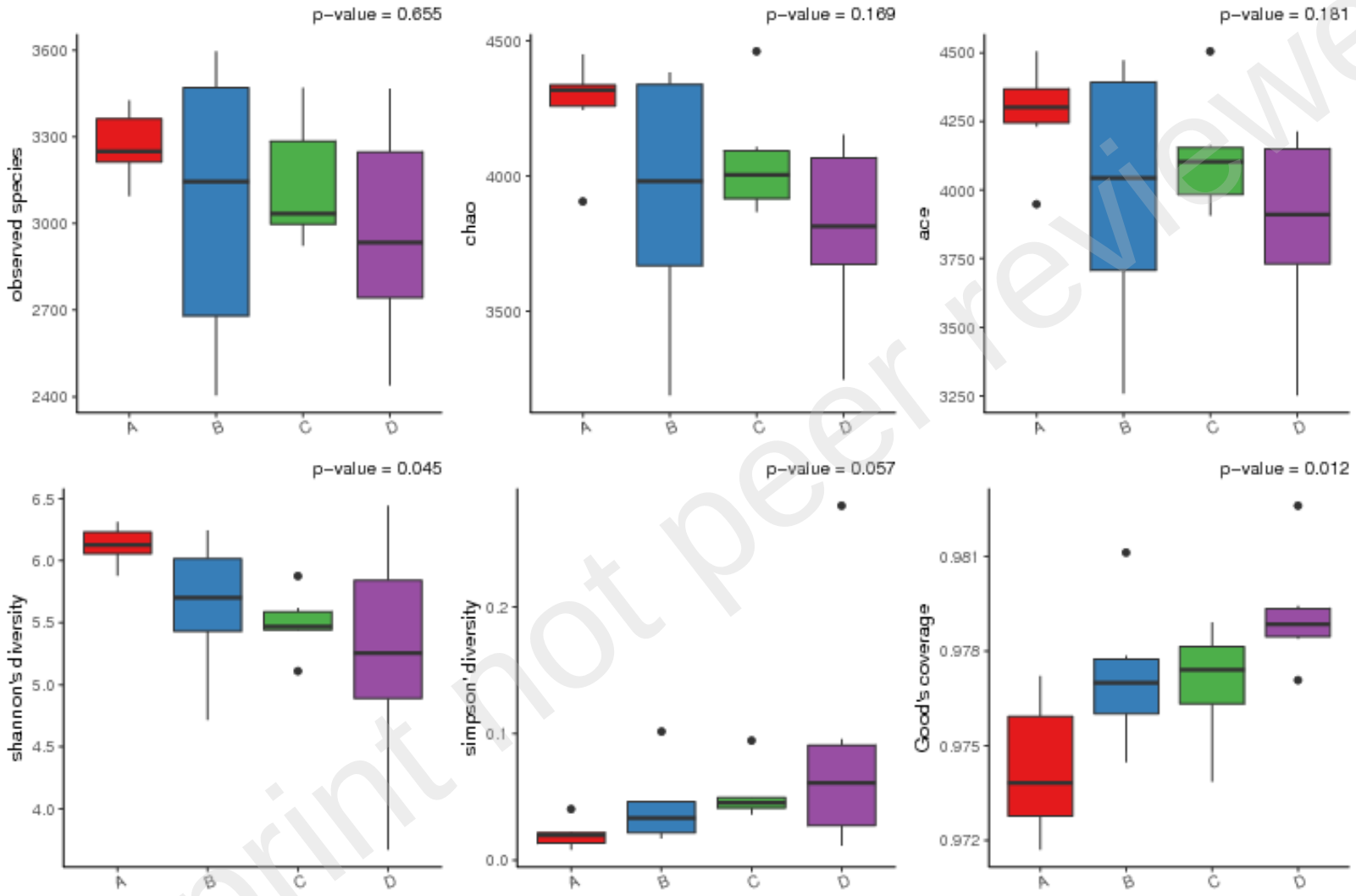


Figure 7. Boxplots of alpha diversity of microbial communities in surface sediment among the mesocosms of the four treatments (A: high N enrichment with crabs; B: high N enrichment without crabs; C: low N enrichment with crabs; D: low N enrichment without crabs).

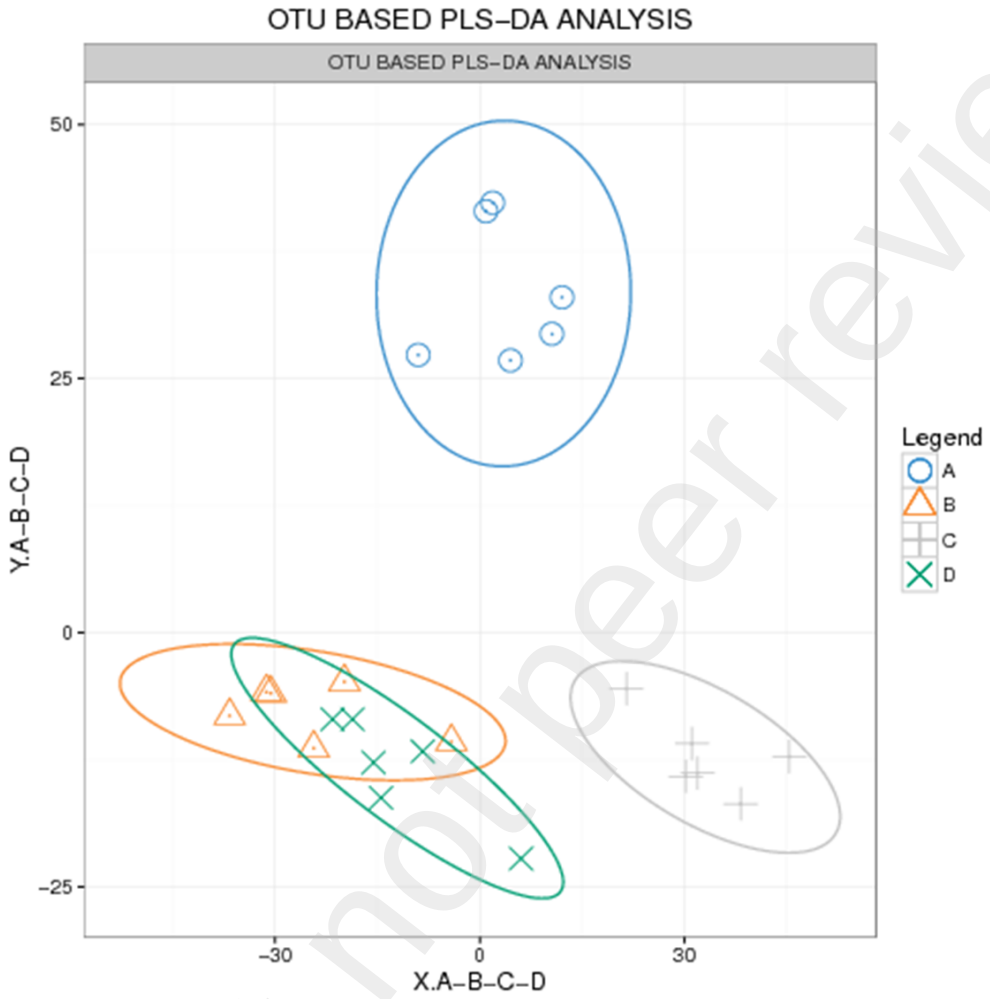
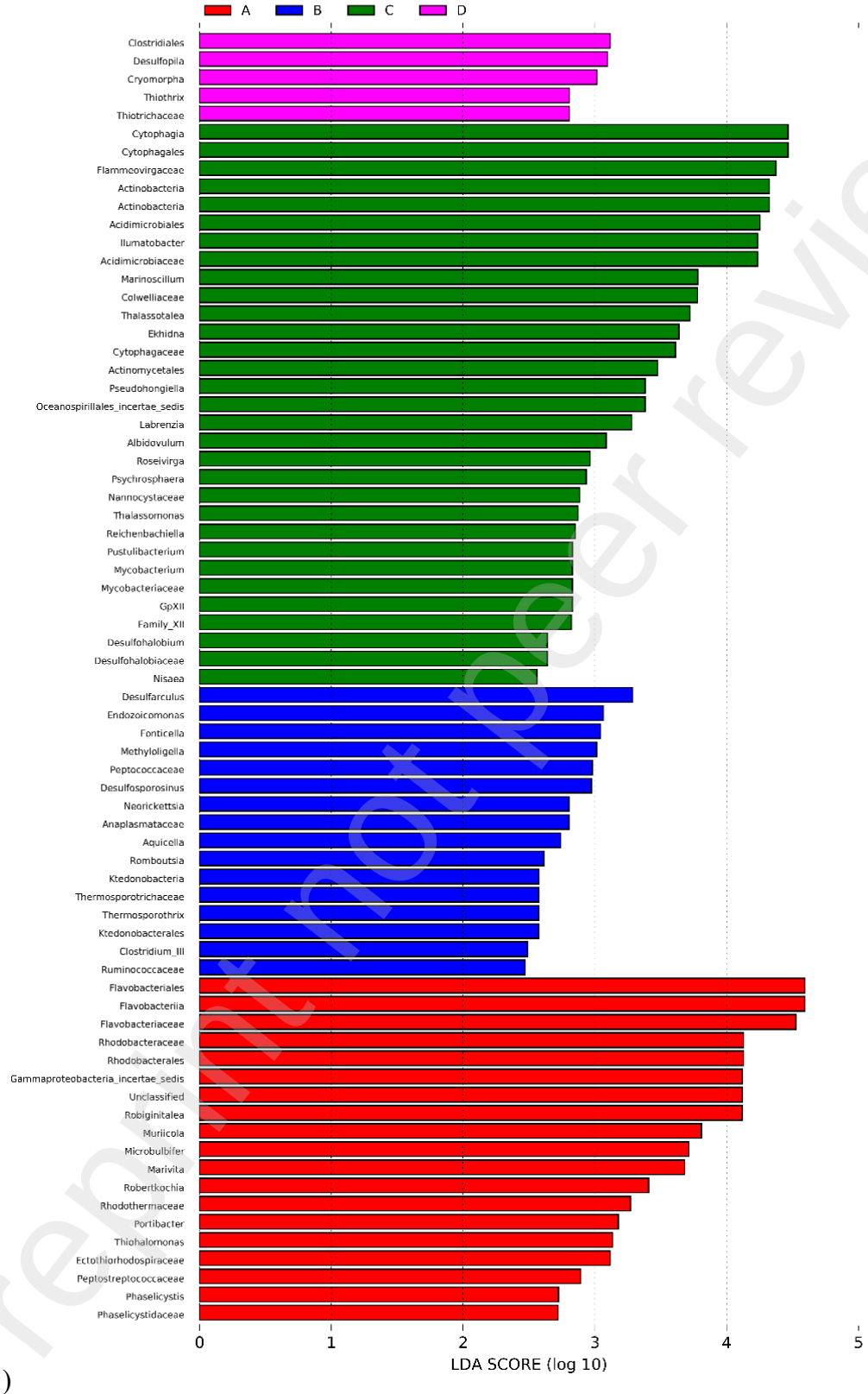


Figure 8. Partial least squares discrimination analysis (PLS-DA) of OTU in surface sediment for the four treatments (A: high N enrichment with crabs; B: high N enrichment without crabs; C: low N enrichment with crabs; D: low N enrichment without crabs).



434 (1)

(2)

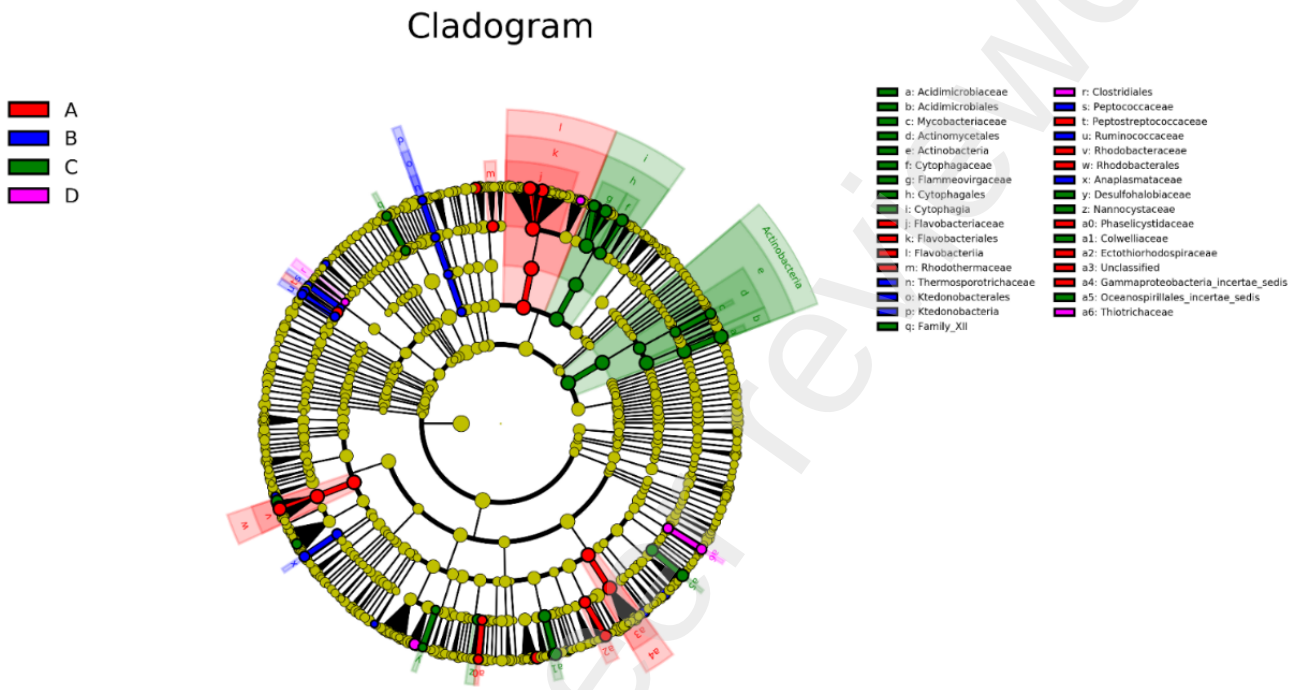
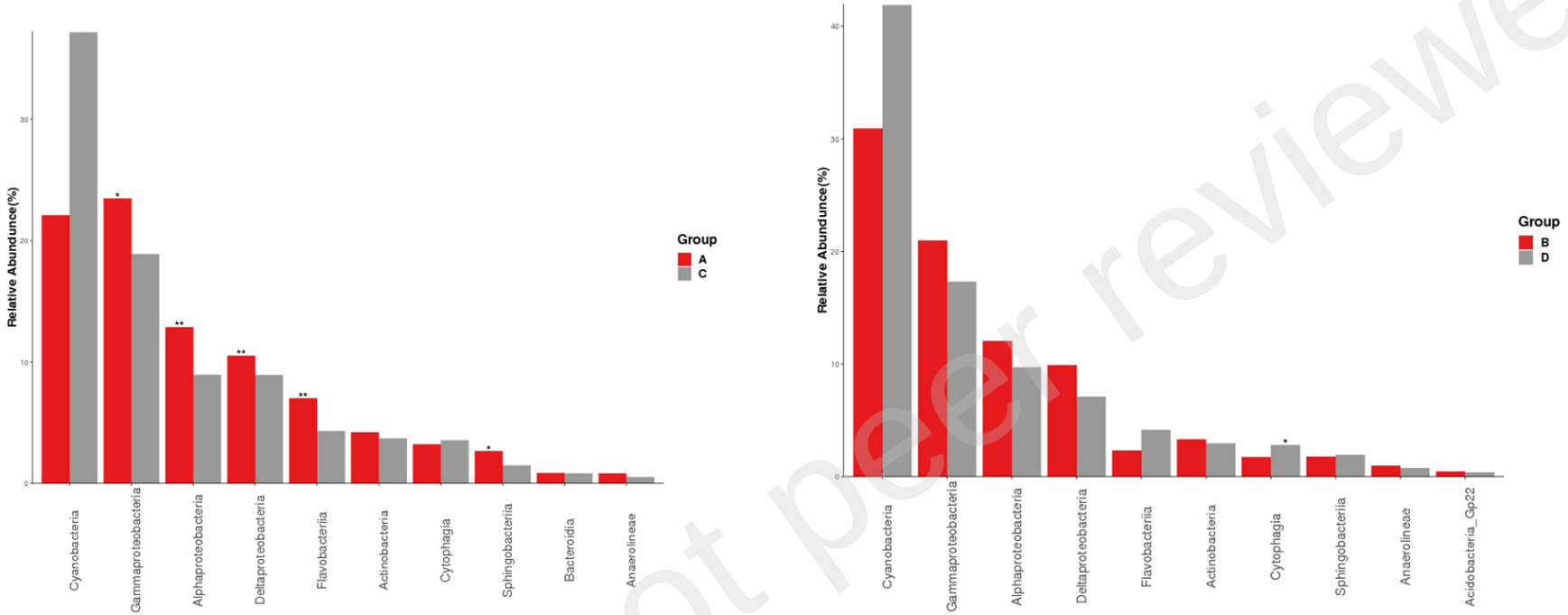


Figure 9. Linear discriminant analysis Effect Size (LEfSe) showing different abundant taxa among samples from the four treatments (A: high N enrichment with crabs; B: high N enrichment without crabs; C: low N enrichment with crabs; D: low N enrichment without crabs). (1) LDA scores; and (2) Cladogram. Node colours represent vital biomarker in the group with same colour as the nodes. Biomarker legends are shown at the top-right corner. Yellow nodes represent unimportant biomarkers in different groups. The six circles from the inner to the outer side represents phylum, class, order, family, genus and species levels, respectively.



445

446 Figure 10. The differences in the relative abundance of the top 10 bacterial classes between the high and low N enrichment mesocosms with
447 crabs (A and C) and high and low N enrichment mesocosms without crabs (B and D) (A: high N enrichment with crabs; B: high N enrichment
448 without crabs; C: low N enrichment with crabs; D: low N enrichment without crabs). Asterisks denote significant difference between treatments
449 (* $p<0.05$; ** $p<0.01$).

Discussion

This study addressed the questions (1) how sesarmid crabs respond to N enrichment by assessing the changes in their food sources and cellulase activity in their hepatopancreas under two contrasting N enrichment levels; and (2) how such changes affect the regulatory role of sesarmid crabs in nutrient dynamics. Our results provide direct experimental confirmations of the hypothesis on the shift of the crabs' diets and also the significant differences in taxonomic and functional structure of the microbiome shaped by the crabs in surface sediment with contracting N enrichment levels.

Effect of N enrichment on MPB

The results of the mesocosm experiment demonstrated that N enrichment (both low and high) significantly changed the composition of the MPB (i.e., increase in the relative abundance of cyanobacteria), increased MPB abundance, at the cost of MPB species richness in the surface sediment. Previous studies have also suggested that MPB community composition can be modified by high nutrient input due to the beneficial effect it has on some species while suppressing others (Hopes and Mock, 2015; Hope et al., 2020). While nutrient may have a regulatory influence on microalgae abundance, other limiting resources seem to control species richness (Balvanera et al., 2006; Burson et al., 2018). Studies from freshwater ecosystems reported that cyanobacteria and chlorophytes often dominate the phytoplankton in eutrophic waters, posing an increasing threat to water quality and global water security (Scheffer and Van Nes, 2007; Richardson et al., 2018). In this study, cyanobacteria became the

overwhelmingly dominant (>90% of total MPB abundance) species in mesocosms with high N enrichment. A number of studies demonstrated that eutrophication leads to widespread hypoxia and anoxia, habitat degradation, alteration of food-web structure, and loss of biodiversity of ecosystems worldwide (Diaz and Rosenberg, 2008; Howarth, 2008; Laurent et al., 2017; Fennel and Testa, 2019). In mangroves, cyanobacteria bloom caused by nutrient enrichment can form mats on mangrove seedlings. This may inhibit the respiration and photosynthesis of leaves inducing the mortality of mangrove seedlings (unpublished observation).

N enrichment effect on sesarmid crabs

In mesocosms with high and low N enrichment, the relative abundance of cyanobacteria was higher in the absence of crabs. Such a finding suggested a potential selective grazing effect on this microalgal group by the crabs. This is consistent with findings from the gut content analysis of *Parasesarma bidens* in the field. This selective grazing effect on cyanobacteria by sesarmid crabs can therefore mitigate eutrophication to some extent, increasing the resilience of the mangrove ecosystems to nutrient enrichment. The stable isotope analysis and the mixing model showed that crabs in mesocosms (both low and high N enrichment) mainly relied on cyanobacteria for both their C and N sources. The leaf ingestion rate of crabs in N-enriched mesocosms indicated that the crabs did not change their leaf-eating habits in a short time (one month). The moulting rate of crabs during the experiment time was higher in mesocosms with high N enrichment, suggesting a higher growth rate in mesocosms

with high N enrichment. This might be because of the higher cyanobacteria abundance in mesocosm with high N enrichment, costing less energy in foraging for the crabs. Although the crabs in both high and low N enrichment mesocosms ingested >50% of the leaves provided, the low ^{13}C enrichment level suggested little carbon assimilation from mangrove leaves. This was consistent with the decreased cellulase activity of the crabs, which limits their capacity to access essential nutrients from different dietary sources (Pavasovic et al., 2004).

Implications for microbial communities and nutrient dynamics

Nitrogen addition to mangrove and marine sediments has been found to decrease microbial biomass (Keuskamp et al., 2015b), bacterial abundance (Luo et al., 2017), and bacterial diversity (Aoyagi et al., 2015). The findings of a recent study by Craig et al. (2021) indicated that nitrogen enrichment has potential implications for carbon and nutrient cycling in mangrove environments due to changes in microbial communities. In our study, the microbial communities in the surface sediment of mesocosms with crabs showed significant differences in species composition and relative abundance between high and low N enrichment. However, no significant difference was found between high and low N enrichment in mesocosms without crabs. The microbial communities also showed significant differences in species composition and relative abundance between mesocosms with and without crabs. Therefore, the individual effect of the presence of crabs or its interaction with N enrichment can have significant effects on microbial communities.

In this study, in mesocosms with crabs, hig/h N enrichment increased Shannon's diversity but decreased Simpson's diversity and Good's coverage compared with low N enrichment. However, in mesocosms without crabs, no differences were found between high and low N enrichment. Actinobacteria and Flavobacteriales were two taxa groups more abundant in mesocosms with crabs. Actinobacteria and Flavobacteriales are saprophytic bacteria using decaying organic matter which contributes to nutrient cycling. The species *Carboxylicivirga mesophile* was also much higher in the mesocosms with crabs than without crabs. Carboxylicivirga was reported as the core genera in the gut of marine invertebrates, such as the mud crab *Scylla paramamosain* (Wei et al., 2019) and the lobster *Homarus gammarus* (Holt et al., 2019). However, their function in the environment or in the host is less known. Five of the top ten bacterial classes showed significantly higher relative abundance in mesocosms (with crabs) of high N enrichment than that of low N enrichment, namely, Gammaproteobacteria, Alphaproteobacteria, Deltaproteobacteria, Flavobacteriia and Sphingobacteriia, which are all related to nutrient dynamics. Gammaproteobacteria is a known fundamental class in marine and coastal ecosystems as they are the major groups involved in N and S cycling (Evans et al., 2008). Gammaproteobacteria contains many environmental strains derived from symbiotic bacteria associated with invertebrates, which were known as sulphur- or methane-oxidizing bacteria that supply energy to their host invertebrates (Distel et al., 1994; Feldman et al., 1997; Mori et al., 2011). There are two distinct microbial strategies in sulfide removal over centimeter-scale distances in coastal sediments, one of which for oxidizing sulfide is carried out

by the Gammaproteobacteria (Teske and Salman, 2014). Several genera of Alphaproteobacteria (e.g., *Methylobacterium* spp.) can metabolize single-carbon compounds. Deltaproteobacteria include a branch of strictly anaerobic genera, which contains most of the known sulphate- and sulphur-reducing bacteria. Sphingobacteriia is a taxonomic class composed of a single order of environmental bacteria that are capable of producing sphingolipids (Boone and Castenholz, 2001). Therefore, the sesarmid crabs can affect nutrient dynamics in surface sediment under eutrophic conditions by modulating functional bacteria groups related to important elemental nutrient cycling (e.g., C, N, and S).

^{13}C and ^{15}N stable isotope labels were applied to trace C and N dynamics in the mesocosms with different treatments. The stable isotope analysis results suggested that both N enrichment and crab presence/absence have a significant effect on C dynamics, but no effect were detected on N dynamics in the surface sediment. Previous studies have suggested that N addition influences microbial community composition and negatively impacts microbially mediated belowground carbon dynamics (Vitousek and Howarth, 1991; Ramirez et al., 2012). The ^{13}C enrichment level of DIC in porewater suggested both N enrichment and crab presence can stimulate C decomposition of mangrove leaf litter. A meta-analysis by Ferreira et al. (2015) found that nutrient enrichment stimulated litter decomposition rate by approximately 50%, and the stimulation was higher in a lower background nutrient concentration. N addition was also found to significantly alter the microbial community and decreased alpha diversity,

556 selecting for taxa that oxidized more complex forms of organic matter (Bulseco et al.,
557 2019). With the combination of metagenomics and biogeochemical rates measurements,
558 evidence was also provided that N addition stimulates microbial respiration in salt
559 marsh sediments (Bulseco et al., 2020).

560 **Implications for the productivity of mangroves**

561 In high N enrichment mesocosms, the presence of crabs increased the primary
562 productivity compared with those without crabs. The main reason might be that in high
563 N enrichment mesocosms, N is not the limiting nutrient anymore and, as MPB can use
564 nutrients more efficiently than the mangrove seedlings, they might compete for other
565 nutrients except for N. In the mesocosms with crabs, the egestion of mangrove leaf litter
566 by crabs and subsequent increased decomposition by functional bacteria can release
567 more nutrients into the sediment, which might mitigate the limitations of other nutrients.
568 However, if the crabs shift their diets to more nutritional foods (e.g., MPB) due to N
569 enrichment and egest less leaf litter, it might decrease the primary productivity of
570 mangroves. Long-term observation in the future is needed to confirm whether the crabs
571 shift their feeding habits (leaf litter consumption rate) under such eutrophic conditions.

572 Evidence from previous large-scale studies suggests that nutrient enrichment effects
573 on ecosystem productivity can differ depending on food web structure. When the
574 structure facilitates efficient energy transfer to higher trophic levels, it can stimulate the
575 production of multiple trophic levels (Davis et al., 2010). Earlier, we found that tight

trophic interactions (within the biogeochemical hotspots created by sesarmid crabs) due to the food selection of sesarmid crabs (e.g., relying on leaf litter and MPB for sources of C and N, respectively) can increase trophic efficiency and thus drive higher nutrient cycling rates and primary productivity in mangroves (Gao et al., under review). Under nutrient enrichment, cyanobacteria became both the main C and N sources of the crabs, which might lead to less of mangrove carbon being transferred to higher trophic levels. In coastal zones, nutrient enrichment reduces trophic transfer efficiencies between algae and primary consumers, generating excess algal production that leads to dead zones (Diaz and Rosenberg, 2008). The decreased diversity in MPB due to N enrichment may also change the trophic interactions between MPB and their grazers. Previous studies suggested that the diversity of MPB is positively related to grazer diversity (Balvanera et al., 2006) and this increased diversity increases overall ecosystem productivity (Worm et al., 2006; Jones et al., 2017). In some low nutrient soft-sediments, nitrogen addition increased the quantity but decreased the quality (decrease proportion of essential fatty acids) of MPB, altering the trophic interactions in the ecosystem, as indicated by a lower contribution of MPB to higher consumers (Hope et al., 2020). N enrichment may alter the functional role of MPB as primary producers and as a basal food resource, leading to a decrease in coastal fisheries (Kritzer et al., 2016). Therefore, N enrichment may also affect secondary productivity, further studies will be needed to see how N enrichment change the trophic interactions and food webs thus affect the secondary productivity of these coastal ecosystems.

Conclusion

N enrichment in tidal water even at a low level can change the composition, abundance and species richness of the mangrove MPB assemblage significantly, leading to a diet shift of sesarmid crabs and a reduced assimilation of C from mangrove leaf litter. The selective grazing effect on MPB by sesarmid crabs may therefore mitigate the effect of eutrophication (decrease in cyanobacteria ratio and increase in MPB species richness) to some extent. However, the diet shift of sesarmid crabs affects the nutrient transfer efficiency to higher trophic levels. These changes might eventually affect leaf consumption by sesarmid crabs in mangroves and compromise their ecosystem role in mediating biogeochemical processes such as mineralization of mangrove production. By directly linking nutrient dynamics (through stable isotope analysis) to the genetic composition of microbial communities, this study provides a framework for achieving mechanistic insights into the biogeochemical processes driven by sesarmid crabs under eutrophic conditions.

Acknowledgements

We thank Hoi Ching Leung and Yan Ping Loo for their assistance with the field investigation and mesocosm experiment. XG was supported by a PhD scholarship awarded by The Chinese University of Hong Kong and a postdoctoral fellowship from The University of Hong Kong. This work was supported by a General Research Fund

(grant number 14302420) awarded to SYL from the Research Grants Council, Hong Kong.

Author contributions

XG and SYL conceived the study and designed the experiments, XG conducted the experiments and performed data analysis supervised by SYL and JDGE. XG, SYL and JDGE wrote the manuscript and gave final approval for publication.

Data availability

Data will be made available on request.

Declarations

Conflicts of interest. The authors declare no conflict of interest.

References

Adachi, K., Toriyama, K., Azekura, T., Morioka, K., Tongnunui, P. and Ikejima, K., 2012. Potent cellulase activity in the hepatopancreas of mangrove crabs. Fisheries Science, 78: 1309-1314.

Ardón, M., Zeglin, L.H., Utz, R.M., Cooper, S.D., Dodds, W.K., Bixby, R.J., Burdett, A.S., Follstad Shah, J., Griffiths, N.A., Harms, T.K. and Johnson, S.L., 2021. Experimental nitrogen and phosphorus enrichment stimulates multiple trophic levels of algal and detrital-based food webs: a global meta-analysis from streams and rivers. Biological Reviews, 96: 692-715.

Balvanera, P., Pfisterer, A.B., Buchmann, N., He, J.S., Nakashizuka, T., Raffaelli, D. and Schmid, B., 2006. Quantifying the evidence for biodiversity effects on ecosystem functioning and services. Ecology Letters, 9: 1146-1156.

639 Baggett, L.P., Heck, K.L., Frankovich, T.A., Armitage, A.R. and Fourqurean, J.W.,
640 2013. Stoichiometry, growth, and fecundity responses to nutrient enrichment by
641 invertebrate grazers in sub-tropical turtle grass (*Thalassia testudinum*)
642 meadows. Marine Biology, 160: 169-180.

643 Barker, M. and Rayens, W., 2003. Partial least squares for discrimination. Journal of
644 Chemometrics: A Journal of the Chemometrics Society, 17: 166-173.

645 Benny, N., Thomas, L.C. and Padmakumar, K.B., 2021. Community structure of
646 microphytobenthos associated with mangrove ecosystems along the southwest
647 coast of India. Estuaries and Coasts, 44:1380-1391.

648 Boldina-Cosqueric, I., Amiard, J.C., Amiard-Triquet, C., Dedourge-Geffard, O.,
649 Métais, I., Mouneyrac, C., Moutel, B. and Berthet, B., 2010. Biochemical,
650 physiological and behavioural markers in the endobenthic bivalve *Scrobicularia*
651 *plana* as tools for the assessment of estuarine sediment quality. Ecotoxicology and
652 Environmental Safety, 73: 1733-1741.

653 Boone D.R., Castenholz R.W. 2001. Bergey's Manual of Systematic Bacteriology.
654 Vol. 1 (The Archaea and the deeply branching and phototrophic Bacteria)
655 (2nd ed.). Springer-Verlag, New York. pp. 465-466.

656 Bricker, S.B., Longstaff, B., Dennison, W., Jones, A., Boicourt, K., Wicks, C. and
657 Woerner, J., 2008. Effects of nutrient enrichment in the nation's estuaries: a decade
658 of change. Harmful Algae, 8: 21-32.

659 Bui, T.H. and Lee, S.Y., 2015. Endogenous cellulase production in the leaf litter
660 foraging mangrove crab *Parasesarma erythodactyla*. Comparative Biochemistry
661 and Physiology Part B: Biochemistry and Molecular Biology, 179: 27-36.

662 Burson, A., Stomp, M., Greenwell, E., Grosse, J. and Huisman, J., 2018. Competition
663 for nutrients and light: testing advances in resource competition with a natural
664 phytoplankton community. Ecology, 99: 1108-1118.

665 Charron, L., Geffard, O., Chaumot, A., Coulaud, R., Jaffal, A., Gaillet, V., Dedourge-
666 Geffard, O. and Geffard, A., 2015. Consequences of lower food intake on the
667 digestive enzymes activities, the energy reserves and the reproductive outcome in
668 *Gammarus fossarum*. PLoS One, 10: p.e0125154.

669 Canfield, D.E., Glazer, A.N. and Falkowski, P.G., 2010. The evolution and future of
670 Earth's nitrogen cycle. Science, 330: 192-196.

671 Cannicci, S., Bartolini, F., Dahdouh-Guebas, F., Fratini, S., Litulo, C., Macia, A.,
672 Mrabu, E.J., Penha-Lopes, G., Paula, J., 2009. Effects of urban wastewater on crab
673 and mollusc assemblages in equatorial and subtropical mangroves of East Africa.
674 Estuarine Coastal and Shelf Science, 84: 305-317.

675 Calow, P. and Sibly, R.M., 1990. A physiological basis of population processes:
676 ecotoxicological implications. Functional Ecology, 283-288.

677 Capdeville, C., Abdallah, K., Buffan-Dubau, E., Lin, C., Azemar, F., Lambs, L.,
678 Fromard, F., Rols, J.L. and Leflaive, J., 2018. Limited impact of several years of
679 pretreated wastewater discharge on fauna and vegetation in a mangrove
680 ecosystem. Marine Pollution Bulletin, 129: 379-391.

681 Capdeville, C., Abdallah, K., Walcker, R., Rols, J.L., Fromard, F. and Leflaive, J.,
682 2019. Contrasted resistance and resilience of two mangrove forests after exposure
683 to long-term and short-term anthropic disturbances. Marine Environmental
684 Research, 146: 12-23.

685 Cross, W.F., Johnson, B.R., Wallace, J.B. and Rosemond, A.D., 2005. Contrasting
686 response of stream detritivores to long-term nutrient enrichment. Limnology and
687 Oceanography, 50: 1730-1739.

688 Davis, J.M., Rosemond, A.D., Eggert, S.L., Cross, W.F. and Wallace, J.B., 2010. Long-
689 term nutrient enrichment decouples predator and prey production. Proceedings of
690 the National Academy of Sciences USA, 107: 121-126.

691 De Coen, W.M. and Janssen, C.R., 1997. The use of biomarkers in *Daphnia magna*
692 toxicity testing. IV. Cellular energy allocation: a new methodology to assess the
693 energy budget of toxicant-stressed Daphnia populations. Journal of Aquatic
694 Ecosystem Stress and Recovery, 6: 43-55.

695 Diaz, R.J. and Rosenberg, R., 2008. Spreading dead zones and consequences for marine
696 ecosystems. Science, 321: 926-929.

697 Douglas, E.J., Pilditch, C.A., Kraan, C., Schipper, L.A., Lohrer, A.M. and Thrush, S.F.,
698 2017. Macrofaunal functional diversity provides resilience to nutrient enrichment
699 in coastal sediments. Ecosystems, 20: 1324-1336.

700 Evans, F.F., Egan, S. and Kjelleberg, S., 2008. Ecology of type II secretion in marine
701 gammaproteobacteria. Environmental microbiology, 10: 1101-1107.

702 Fennel, K. and Testa, J.M., 2019. Biogeochemical controls on coastal hypoxia. Annual
703 Review of Marine Science, 11: 105-130.

704 Ferreira, V., Castagneyrol, B., Koricheva, J., Gulis, V., Chauvet, E. and Graça, M.A.,
705 2015. A meta-analysis of the effects of nutrient enrichment on litter decomposition
706 in streams. Biological Reviews, 90: 669-688.

707 Fitch, J.E., and T.P. Crowe. 2012. Combined effects of inorganic nutrients and organic
708 enrichment on intertidal benthic macrofauna: An experimental approach. Marine
709 Ecology Progress Series 461: 59–70.

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P. and Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science, 320: 889-892.

Gao, X. and Lee, S.Y., 2022. Feeding strategies of mangrove leaf-eating crabs for meeting their nitrogen needs on a low-nutrient Diet. Frontiers in Marine Science, 9, p.872272.

Gruber, N. and Galloway, J.N., 2008. An Earth-system perspective of the global nitrogen cycle. Nature, 451: 293-296.

Guignard, M.S., Leitch, A.R., Acquisti, C., Eizaguirre, C., Elser, J.J., Hessen, D.O., Jeyasingh, P.D., Neiman, M., Richardson, A.E., Soltis, P.S. and Soltis, D.E., 2017. Impacts of nitrogen and phosphorus: from genomes to natural ecosystems and agriculture. Frontiers in Ecology and Evolution, 5, p.70.

He, Q. and Silliman, B.R., 2015. Biogeographic consequences of nutrient enrichment for plant-herbivore interactions in coastal wetlands. Ecology Letters, 18: 462-471.

Holt, C.C., van der Giezen, M., Daniels, C.L., Stentiford, G.D. and Bass, D., 2020. Spatial and temporal axes impact ecology of the gut microbiome in juvenile European lobster (*Homarus gammarus*). The ISME journal, 14: 531-543.

Hopes, A. and Mock, T., 2015. Evolution of microalgae and their adaptations in different marine ecosystems. eLS, 1-9.

Hope, J.A., Paterson, D.M. and Thrush, S.F., 2020a. The role of microphytobenthos in soft-sediment ecological networks and their contribution to the delivery of multiple ecosystem services. Journal of Ecology, 108: 815-830.

Hope, J.A., Hewitt, J., Pilditch, C.A., Savage, C. and Thrush, S.F., 2020b. Effect of nutrient enrichment and turbidity on interactions between microphytobenthos and a key bivalve: implications for higher trophic levels. Frontiers in Marine Science, 7, p.695.

Howarth, R.W., 2008. Coastal nitrogen pollution: a review of sources and trends globally and regionally. Harmful Algae, 8: 14-20.

Hulot, F.D., Lacroix, G., Lescher-Moutoue, F. and Loreau, M., 2000. Functional diversity governs ecosystem response to nutrient enrichment. Nature, 405: 340-344.

Isbell, F., Reich, P.B., Tilman, D., Hobbie, S.E., Polasky, S. and Binder, S., 2013. Nutrient enrichment, biodiversity loss, and consequent declines in ecosystem

productivity. Proceedings of the National Academy of Sciences USA, 110: 11911-11916.

Jesus, B., Mendes, C.R., Brotas, V. and Paterson, D.M., 2006. Effect of sediment type on microphytobenthos vertical distribution: Modelling the productive biomass and improving ground truth measurements. Journal of Experimental Marine Biology and Ecology, 332: 60-74.

Jones, H.F., Pilditch, C.A., Hamilton, D.P. and Bryan, K.R., 2017. Impacts of a bivalve mass mortality event on an estuarine food web and bivalve grazing pressure. New Zealand Journal of Marine and Freshwater Research, 51: 370-392.

Koehn, R.K. and Bayne, B.L., 1989. Towards a physiological and genetical understanding of the energetics of the stress response. Biological Journal of the Linnean Society, 37: 157-171.

Kozich, J.J., Westcott, S.L., Baxter, N.T., Highlander, S.K. and Schloss, P.D., 2013. Development of a dual-index sequencing strategy and curation pipeline for analyzing amplicon sequence data on the MiSeq Illumina sequencing platform. Applied and environmental microbiology, 79: 5112-5120.

Kremen, C., 2005. Managing ecosystem services: what do we need to know about their ecology? Ecology Letters, 8: 468-479.

Kristensen E., 2008. Mangrove crabs as ecosystem engineers: with emphasis on sediment processes. Journal of Sea Research, 59: 30-43.

Kritzer, J.P., DeLucia, M.B., Greene, E., Shumway, C., Topolski, M.F., Thomas-Blate, J., Chiarella, L.A., Davy, K.B. and Smith, K., 2016. The importance of benthic habitats for coastal fisheries. BioScience, 66: 274-284.

Laurent, A., Fennel, K., Cai, W.J., Huang, W.J., Barbero, L. and Wanninkhof, R., 2017. Eutrophication-induced acidification of coastal waters in the northern Gulf of Mexico: Insights into origin and processes from a coupled physical-biogeochemical model. Geophysical Research Letters, 44: 946-956.

Lee, S.Y., 1989. The importance of sesarminae crabs *Chiromanthes* spp. and inundation frequency on mangrove (*Kandelia candel* (L.) Druce) leaf litter turnover in a Hong Kong tidal shrimp pond. Journal of Experimental Marine Biology and Ecology, 131: 23-43.

Lee, S.Y. 1995. Cheliped size and structure: the evolution of a multi-functional decapod organ. Journal of Experimental Marine Biology and Ecology, 193: 161-176.

Lee S.Y., 2008. Mangrove macrobenthos: assemblages, services, and linkages. Journal of Sea Research, 59: 16-29.

779 Lee, S.Y., 2016. From blue to black: Anthropogenic forcing of carbon and nitrogen
780 influx to mangrove-lined estuaries in the South China Sea. Marine Pollution
781 Bulletin, 109: 682-690.

782 MacDonnell, C.P., Zhang, L., Griffiths, L. and Mitsch, W.J., 2017. Nutrient
783 concentrations in tidal creeks as indicators of the water quality role of mangrove
784 wetlands in Southwest Florida. Ecological indicators, 80: 316-326.

785 McQueen, D.J., Johannes, M.R., Post, J.R., Stewart, T.J. and Lean, D.R., 1989.
786 Bottom-up and top-down impacts on freshwater pelagic community
787 structure. Ecological Monographs, 59: 289-309.

788 Mégevand, L., Martínez-Alarcón, D., Theuerkauff, D., Rivera-Ingraham, G.A.,
789 Lejeune, M., Lignot, J.H. and Sucré, E., 2021. The hepatopancreas of the
790 mangrove crab *Neosarmatium africanum:* a possible key to understanding the
791 effects of wastewater exposure (Mayotte Island, Indian Ocean). Environmental
792 Science and Pollution Research, 28: 60649-60662.

793 Moore, J.W. and Semmens, B.X., 2008. Incorporating uncertainty and prior
794 information into stable isotope mixing models. Ecology Letters, 11: 470-480.

795 Morris, L., and M.J. Keough. 2003. Variation in the response of intertidal infaunal
796 invertebrates to nutrient additions: Field manipulations at two sites within port
797 Phillip Bay, Australia. Marine Ecology Progress Series, 250: 35-49.

798 Nessel, M. P., Konnovitch, T., Romero, G. Q., and González, A. L., 2021. Nitrogen and
799 phosphorus enrichment cause declines in invertebrate populations: a global
800 meta-analysis. Biological Reviews, 96: 2617-2637.

801 Nijssen, M.E., WallisDeVries, M.F. and Siepel, H., 2017. Pathways for the effects of
802 increased nitrogen deposition on fauna. Biological Conservation, 212: 423-431.

803 Nixon, S.W., 1998. Enriching the sea to death. Scientific American, 9: 48-53.

804 Ouyang, X., Guo, F., 2016. Paradigms of mangroves in treatment of anthropogenic
805 wastewater pollution. Science of the Total Environment, 544: 971–979.

806 Paerl, H.W., Valdes, L.M., Joyner, A.R., Piehler, M.F. and Lebo, M.E., 2004. Solving
807 problems resulting from solutions: evolution of a dual nutrient management
808 strategy for the eutrophying Neuse River Estuary, North Carolina. Environmental
809 Science & Technology, 38: 3068-3073.

810 Palumbi, S.R., Sandifer, P.A., Allan, J.D., Beck, M.W., Fautin, D.G., Fogarty, M.J.,
811 Halpern, B.S., Incze, L.S., Leong, J.A., Norse, E. and Stachowicz, J.J., 2009.
812 Managing for ocean biodiversity to sustain marine ecosystem services. Frontiers
813 in Ecology and the Environment, 7: 204-211.

814 Pavasovic, M., Richardson, N.A., Anderson, A.J., Mann, D. and Mather, P.B., 2004.
815 Effect of pH, temperature and diet on digestive enzyme profiles in the mud crab,
816 *Scylla serrata*. Aquaculture, 242: 641-654.

817 Penha-Lopes, G., Bartolini, F., Limbu, S., Cannicci, S., Kristensen, E. and Paula, J.,
818 2009. Are fiddler crabs potentially useful ecosystem engineers in mangrove
819 wastewater wetlands? Marine Pollution Bulletin, 58: 1694-1703.

820 Peterson, B.J., Deegan, L., Helfrich, J., Hobbie, J.E., Hullar, M., Moller, B., Ford, T.E.,
821 Hershey, A., Hiltner, A., Kipphut, G. and Lock, M.A., 1993. Biological responses
822 of a tundra river to fertilization. Ecology, 74: 653-672.

823 Posey, M.H., T.D. Alphin, and L. Cahoon., 2006. Benthic community responses to
824 nutrient enrichment and predator exclusion: Influence of background nutrient
825 concentrations and interactive effects. Journal of Experimental Marine Biology
826 and Ecology, 330: 105-118.

827 Prather, C.M., Pelini, S.L., Laws, A., Rivest, E., Woltz, M., Bloch, C.P., Del Toro, I.,
828 Ho, C.K., Kominoski, J., Newbold, T.S. and Parsons, S., 2013. Invertebrates,
829 ecosystem services and climate change. Biological Reviews, 88: 327-348.

830 Ramirez, K.S., Craine, J.M. and Fierer, N., 2012. Consistent effects of nitrogen
831 amendments on soil microbial communities and processes across biomes. Global
832 change biology, 18: 1918-1927.

833 Richardson, J., Feuchtmayr, H., Miller, C., Hunter, P.D., Maberly, S.C. and Carvalho,
834 L., 2019. Response of cyanobacteria and phytoplankton abundance to warming,
835 extreme rainfall events and nutrient enrichment. Global Change Biology, 25:
836 3365-3380.

837 Scheffer, M., Carpenter, S., Foley, J.A., Folke, C. and Walker, B., 2001. Catastrophic
838 shifts in ecosystems. Nature, 413: 591-596.

839 Scheffer, M. and Nes, E.H.V., 2007. Shallow lakes theory revisited: various alternative
840 regimes driven by climate, nutrients, depth and lake size. In: Shallow Lakes in a
841 Changing World. Springer, Dordrecht. pp. 455-466.

842 Silva, I. C., and Paula, J. 2008. Is there a better chela to use for geometric morphometric
843 differentiation in brachyuran crabs? A case study using *Pachygrapsus marmoratus*
844 and *Carcinus maenas*. Journal of the Marine Biological Association of the United
845 Kingdom, 88: 941-953.

846 Vitousek, P.M. and Howarth, R.W., 1991. Nitrogen limitation on land and in the sea:
847 how can it occur? Biogeochemistry, 13: 87-115.

Wear, R.J. and Tanner, J.E., 2007. Spatio-temporal variability in faunal assemblages surrounding the discharge of secondary treated sewage. Estuarine, Coastal and Shelf Science, 73: 630-638.

Wei, H., Wang, H., Tang, L., Mu, C., Ye, C., Chen, L. and Wang, C., 2019. High-throughput sequencing reveals the core gut microbiota of the mud crab (*Scylla paramamosain*) in different coastal regions of southern China. BMC genomics, 20: 1-12.

Worm, B., Barbier, E.B., Beaumont, N., Duffy, J.E., Folke, C., Halpern, B.S., Jackson, J.B., Lotze, H.K., Micheli, F., Palumbi, S.R. and Sala, E., 2006. Impacts of biodiversity loss on ocean ecosystem services. Science, 314: 787-790.

Tam, N.F.Y., Wong, A.H.Y., Wong, M.H. and Wong, Y.S., 2009. Mass balance of nitrogen in constructed mangrove wetlands receiving ammonium-rich wastewater: Effects of tidal regime and carbon supply. Ecological Engineering, 35: 453-462.

Theuerkauff, D., Rivera-Ingraham, G.A., Mercky, Y., Lejeune, M., Lignot, J.H. and Sucré, E., 2018. Effects of domestic effluent discharges on mangrove crab physiology: integrated energetic, osmoregulatory and redox balances of a key engineer species. Aquatic Toxicology, 196: 90-103.

Theuerkauff, D., Rivera-Ingraham, G.A., Lambert, S., Mercky, Y., Lejeune, M., Lignot, J.H. and Sucré, E., 2020. Wastewater bioremediation by mangrove ecosystems impacts crab ecophysiology: In-situ caging experiment. Aquatic Toxicology, 218.

Teske, A., and V. Salman. 2014. The family Beggiatoaceae, In E. Rosenberg, D. L. EF, S. Lory, E. Stackebrandt, and F.Thompson [eds.], The prokaryotes: Gammaproteobacteria. Springer. p. 93-134.

Villnäs, A., Norkko, J., Hietanen, S., Josefson, A.B., Lukkari, K. and Norkko, A., 2013. The role of recurrent disturbances for ecosystem multifunctionality. Ecology, 94: 2275-2287.