1   **High Temperature and Crab Density Reduce Atmospheric Nitrogen**

2   **Fixation in Red Sea Mangrove Sediments**

3

4   Maryam S. Qashqari[1+], Neus Garcias-Bonet[1+*], Marco Fusi[2,3], Jenny M. Booth[2],

5   Daniele Daffonchio[2], and Carlos M. Duarte[1]

6

7   [1]Red Sea Research Center and Computational Bioscience Research Center, Division of

8   Biological and Environmental Sciences and Engineering, King Abdullah University of

9   Science and Technology (KAUST), Thuwal, 23955-6900, Saudi Arabia.

10   [2]Red Sea Research Center, Division of Biological and Environmental Sciences and

11   Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal,

12   23955-6900, Saudi Arabia.

13   [3]Edinburgh Napier University, School of Applied Sciences, Edinburgh, UK.

14

15

16   [+]**Equal contribution**

17   [*]**Corresponding author**: Garcias-Bonet, Neus.

18   **Email**: neus.garciasbonet@kaust.edu.sa, n.garcias.bonet.ng@gmail.com

19

22 **Abstract**

23   Mangrove ecosystems are highly productive and provide important ecosystem

24 services. However, in the Red Sea mangroves are under severe nutrient-limiting

25 conditions, reflected in dwarf plants. The nutrient limitation is especially acute for iron, as

26 verified experimentally, although the low carbon-to-nutrient stoichiometric ratios

27 reported for Red Sea mangrove leaves are indicative of general nutrient depletion.

28 Therefore, atmospheric nitrogen (N_2) fixation in mangrove sediments might be

29 particularly important considering the minimal nitrogen inputs from land. Here, we tested

30 the effect of temperature and crab density on sediment N_2 fixation rates in mature and

31 juvenile mangrove (*Avicennia marina*) stands in the central Red Sea. The average N_2

32 fixation rates (from 0.002 ± 0.002 to 0.46 ± 0.12 mg N m^{-2} d^{-1}) fall in the low range of N_2

33 fixation rates reported in mangroves elsewhere, which is in agreement with the small size

34 of the mangrove plants. Mature mangrove sediments hold higher N_2 fixation rates than

35 the juvenile mangrove sediment, related to a higher sediment organic matter and carbon

36 content. We found a detrimental effect of temperature and crab density on sediment N_2

37 fixation rates. Maximum N_2 fixation rates were detected at 28°C with a sharp decrease at

38 35°C. Similarly, high crab-density reduced N_2 fixation, likely due to the sediment

39 oxygenation or the grazing of cyanobacteria by crabs. This is supported by i) previously

40 reported higher oxygen concentration and redox around burrows compared to undisturbed

41 sediment and ii) lighter sediment carbon isotopic composition in high crab-density than in

42 low crab-density sediments, indicating a higher contribution of microphytobenthos in the

43 mature sediments supporting low crab-density. Our data document temperature and crab

44 density as factors affecting N_2 fixation in the Red Sea mangrove sediments.

45

1. Introduction

Mangroves are highly productive ecosystems occupying the upper intertidal zone in the land-sea interface of tropical and subtropical regions (Giri et al. 2011). They provide important ecosystem services, being habitat for a wide range of living organisms such as birds, crabs, and fish (Polidoro et al. 2010; Reef, Feller, and Lovelock 2010), protecting the coast from erosion due to exposure to tides, storms, and currents (Mazda et al. 1997), providing wood for construction and fuel (Walters et al. 2008), and acting as intense carbon sinks (Bouillon et al. 2008). Due to their large carbon storage capacity, mangroves rank among the most carbon-rich forests in the tropics (Donato et al. 2011).

A significant part of mangrove biomass is exported to adjacent coastal areas, as litter and particulate and dissolved organic matter, or stored in sediments. At the same time, mangroves receive organic matter from adjacent coastal areas by tides and rivers. The magnitude of these fluxes is highly variable (Lee 1995; Kristensen et al. 2008). However, the continuous export and sink of nutrients could lead to nutrient limitation for plant growth and production. As they often grow in river deltaic areas, they receive high inputs of nutrients and sediment that contribute to maintain a positive nutrient balance required to support high primary production, nutrient sequestration in sediments and export to adjacent marine habitats (Jennerjahn and Ittekkot 2002). However, nutrient supply to mangrove stands is restricted in arid and/or karstic areas, where freshwater runoff and the associated nutrient and sediment delivery is limited, often resulting in dwarf, nutrient-limited mangrove trees (Almahasheer, Duarte, and Irigoien 2016a, 2016b).

Central Red Sea mangroves are stunted and severely nutrient-limited. Experimental nutrient additions showed that they are iron-limited (Almahasheer, Duarte, and Irigoien

3

69   2016a) due to absence of inputs from land and low nutrient concentration in Red Sea

70   seawater (Mandura 1997; Saifullah 1997). Iron is a co-factor in the nitrogenase enzyme

71   (Howard and Rees 1996), and its deficiency limits atmospheric nitrogen (N_2) fixation, a

72   key process for nitrogen (N) supply, particularly where terrestrial inputs are minimal.

73   *Avicennia marina* is the most abundant mangrove species (El-Juhany 2009) in the 135

74   km^2 mangrove forest of the Red Sea (Almahasheer, Duarte, and Irigoien 2016a) and,

75   considering the low nutrient inputs to the area, N_2 fixation could be an essential N source

76   to support its growth. Recent studies in the Red Sea pointed out an increasing importance

77   of N_2 fixation as a source of N in mangrove ecosystems towards the north, based on the

78   stable N isotopic composition of mangrove leaves and sediments (Duarte et al. 2018;

79   Garcias-Bonet et al. 2019a). However, to the best of our knowledge, actual N_2 fixation

80   rates in Red Sea mangrove sediments have not been estimated yet.

81   N_2 fixation has been detected in sediments, roots, rhizosphere and decomposing

82   leaves in mangrove systems elsewhere and represents, therefore, a key, rate-limiting step

83   in the N cycle (Alongi et al. 2002; Ray et al. 2014). It also occurs in large cyanobacterial

84   mats that typically cover mangrove sediments and contributes to mangroves primary

85   productivity (Alvarenga et al. 2015). High concentrations of calcium, carbon, magnesium,

86   nitrogen, phosphorus, and sulphur, as well as several soluble extracellular carbohydrates,

87   have been detected in cyanobacterial mats providing nutrients to mangroves (Lovelock et

88   al. 2010). A substantial heterotrophic bacterial community exploits this capacity by

89   colonizing and producing strong attachments to the sheaths and mucilage of some

90   cyanobacteria (Simmons et al. 2008).

4

91   Mangrove ecosystems support abundant fauna (Nagelkerken et al. 2008), whose

92   activity alter the sediment structure, chemical composition and affect biogeochemical

93   processes (Aller 1994; Kristensen 2008; Bertics et al. 2010). Macrobenthic animals such

94   as crabs, shrimps and mollusks bioturbate the sediment by excavating galleries, thereby

95   increasing oxygen supply and oxidation of sediment materials (Laverock et al. 2011), and

96   by feeding on the first layer of the sediment, which removes microbes, algae and

97   meiobenthic fauna (Reinsel 2004).

98   The effect of bioturbators on N_2 fixation in mangrove soils has not been extensively

99   studied. Available data provide evidence that sediment aeration inhibits nitrogenase

100   enzyme activity (Goldberg, Nadler, and Hochman 1987; Bertics et al. 2010). However,

101   oxygen inputs also oxidize iron sulfide produced in anoxic sediments, favoring the release

102   of iron and its uptake in the presence of organic ligands (Luther et al. 1992). Indeed,

103   bioturbators have been reported to enhance metal, including iron, release (Boto and

104   Robertson 1990). In Brazilian mangroves iron sulfide oxidation has been reported to be

105   enhanced under elevated temperatures, when mangrove evapotranspiration is highest, and

106   increased crab activity (*Uca* spp.), which leads to release of iron from pyrite (Ferreira et

107   al. 2007). As iron is a limiting nutrient in the Central Red Sea and required to synthesize

108   nitrogenase, aeration of sediments through bioturbation may enhance iron mobilization

109   and, therefore, nitrogenase synthesis. However, crab feeding on cyanobacterial mats may

110   reduce N_2 fixation.

111   In addition to bioturbators, temperature maybe an important driver of N_2 fixation in

112   Red Sea mangrove sediments, as it is considered the warmest of all seas, reaching up to

113   35°C in summer (Burkholz et al. 2019; Chaidez et al. 2017). There is growing evidence

5

114   that N_2 fixation is temperature-dependent, with maximum rates typically achieved at

115   about 25°C across ecosystems (20 to 30°C, (Houlton et al. 2008; Zhou et al. 2016)). This

116   is consistent with findings for seagrass in the Red Sea (Garcias-Bonet et al. 2018) and the

117   Mediterranean Sea (Garcias-Bonet et al. 2019b), showing declining N_2 fixation rates at

118   temperatures > 30°C. Hence, this suggests that N_2 fixation in Red Sea mangrove forests

119   can also be suppressed at temperatures > 30°C.

120   Based on these premises, here we aim to determine if temperature and crab density

121   influence N_2 fixation rates in *Avicennia marina* mangrove sediments in the central Red

122   Sea coast. In particular, we hypothesize that: i) N_2 fixation is suppressed at high

123   temperature, and ii) high crab-density affects N_2 fixation, although the effects may vary

124   from negative, where crabs remove N-fixing microorganism and therefore reduce N_2

125   fixation in mangroves, to positive where bioturbation increases nutrient supply to support

126   N_2 fixation, to neutral when both effects cancel each other. To test our hypothesis, we

127   experimentally set up plots with different bioturbation levels by manipulating the density

128   of *Cranuca inversa* and *Dotilla sulcata* crab species, in a mature and a juvenile mangrove

129   stand and we measured N_2 fixation rates in the first 10 cm of mangrove sediments at two

130   *in situ* temperatures (28°C and 35°C). We note that our measurements are not designed to

131   resolve all components of N_2 fixation in the mangrove ecosystems, as we do not consider

132   N_2 fixation associated with mangrove roots (Alfaro-Espinoza and Ullrich 2015) or leaf

133   litter (Pelegri, Rivera-Monroy, and Twilley 1998), as these would differ greatly between

134   plots supporting mature trees and young seedlings.

135   **2. Material and methods**

136   **2.1. Study site**

137   The study site is located at the 'Ibn Sina field research station and nature

138   conservation area' in KAUST (Thuwal, Saudi Arabia, 22° 20' 25.032'' N; 39° 5'

139   17.411''E). This site is a natural mangrove fringe entirely vegetated by *A. marina* species

140   and supports a high density of ecosystem engineering burrowing crabs, *C. inversa* and *D.*

141   *sulcata*. Two experimental sites were chosen, one colonized by natural mature trees of *A.*

142   *marina* (mature stand) and the second colonized by one-year old planted propagules of *A.*

143   *marina*. (juvenile stand). For establishment of the juvenile stand, propagules, collected

144   from the same location, were grown in a nursery in autochthonous sediment.

145   Subsequently, one-month old seedlings were transplanted into the mangrove study site.

146   To evaluate the effect of crab density on sediment N_2 fixation rates, in each

147   experimental site (mature and juvenile stands) we randomly selected 5 plots of 4 m^2 (2×2

148   m), which we enriched with *C. inversa* and *D. sulcata* crabs (high crab-density mangrove

149   plots) and 5 plots of 4 m^2 (2×2 m) with natural crab densities (low crab-density mangrove

150   plots). One year prior to performing the experiment, we created plots artificially enriched

151   with these two species of crabs, continuously surveying crab numbers following Skov et

152   al. (2002). Throughout the study period the crab densities in the low crab-density plots of

153   the mature and juvenile mangrove stands were 3.3 ± 1.1 and 11.5 ± 0.6 crabs m^{-2}

154   (average ± SEM), respectively, while in the high crab-density plots of the mature and

155   juvenile mangrove stands, i.e. enriched in crabs, crab densities were 17.6 ± 5.5 and 32.6 ±

156   1.6 crabs m^{-2} (average ± SEM), respectively (see Fig. S1 for details). The size of crab

157   burrows was on average 1 cm in diameter × 5 cm in depth, with a determined 10 cm

158   diameter halo of influence resulting in an area of 78.5 cm^2 (Booth et al. 2019). The

159   enrichment approach was adopted for two main reasons. Firstly, it is almost impossible to

160   effectively remove crabs from sediment without altering the structure, since it requires

161   digging up at least the first 20 cm of sediment, compromising the N-fixing microbiome

162   object of this study. Secondly, crab enrichment is effective due to the high burrow fidelity

163   of these animals which tend to maintain the same burrow if the environmental conditions

164   are favorable (Booth et al. 2019). Crab activity was monitored before the experiment by

165   manually plugging the burrow with a thin layer of sediment and waiting for 30 minutes.

166   Active crabs quickly remove this layer of sediment to exit and forage on the surrounding

167   area. Therefore, the number of burrows opened indicates how many crabs were

168   effectively active (Andreetta et al. 2014).

169   We performed two independent assessments of N_2 fixation rates in sediments

170   collected on experimental plots: the first sampling was performed in

171   November/December 2016 when seawater temperature was 35°C, and the second was

172   performed in January/February 2017 when seawater temperature was 28°C. Based on

173   prior research in the Red Sea (Garcias-Bonet et al. 2018) and elsewhere (Houlton et al.

174   2008; Zhou et al. 2016; Garcias-Bonet et al. 2019b), we expected N_2 fixation at 35°C,

175   which corresponds to the maximum water temperature observed in the Central Red Sea

176   (Chaidez et al. 2017), to be low, compared to those rates at 28°C. These temperature

177   treatments were guided by previous results in seagrass sediments close to our study site,

178   which showed maximum N_2 fixation rates at 28°C and minimum rates at 33°C, the

179   maximum temperature tested (Garcias-Bonet et al. 2018). The *in situ* air and sediment

180   temperature were measured and continuously recorded every 15 minutes by an on-site

181   weather station (Ibimet, Florence, Italy). Sediment temperature was measured at the

182   sediment surface and 15 cm below ground. The *in situ* seawater salinity was measured

183   from 13 November to 12 December 2016 (corresponding to the first sampling time) and

184   from 28 January to 27 February 2017 (corresponding to the second sampling time) by

185   deploying an EXO1 multiparameter probe (Xylem Inc., USA) at the closest distance from

186   the mangrove edge where it remained completely submerged. Salinity was measured and

187   recorded every 5 minutes for two periods of one month each.

188   **2.2.   Sediment characterization**

189   The organic matter (OM) content in mangrove sediments was calculated by loss on

190   ignition (Dean 1974). The sediment organic carbon (C) and nitrogen (N) contents were

191   measured using a CHNS Elemental Analyzer (Flash 2000, KAUST analytical core

192   laboratory, Saudi Arabia). Sediment samples were dried, ground and acidified before the

193   analysis to remove carbonates. Sediment OM, C, and N contents were measured on five

194   replicates per treatment. Sediment C and N isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) was

195   analyzed on five replicates per treatment only for samples collected in January and

196   February 2017, when seawater temperature was 28°C. Dried and ground sediment

197   samples were acidified and analyzed using an isotope ratio mass spectrometer (Thermo-

198   Finnegan Delta V IRMS, UH-Hilo Analytical laboratory, Hawaii). Results of the $\delta^{13}C$ and

199     $\delta^{15}N$ isotopic analysis are reported in parts per thousand (‰) and Vienna Pee Dee

200   Belemnite limestone (V-PDB) was used as a standard for the stable C isotope and

201   atmospheric N_2 for the stable N isotope.

202   **2.3.   Sediment N_2 fixation rates**

203   N_2 fixation rates in mangrove sediment samples were estimated using the acetylene

204   reduction assay (ARA) (Boto and Robertson 1990; Capone 1993; Howard and Rees

205   1996). The sediments were sampled during high tide using cores (cylindrical Plexiglas

206 cores of 10.4 cm in diameter and 27 cm in height) to avoid disruption of the sediment

207 structure. For each sampling and sediment type, five replicated cores were collected from

208 random positions in high and low crab-density plots in mature and juvenile mangrove

209 stands and immediately transported to the laboratory and processed. The sediment

210 samples were extracted from the cores and processed by slicing 10 cm from the surface.

211 Sediment samples (200 ml of the first 10 cm of sediment) were placed in 500 ml Pyrex

212 glass incubation jars and amended with 80 ml of seawater collected from the same

213 sampling location. The Pyrex jars containing the sediment slurry were closed with lids

214 equipped with a gas-tight valve to allow sampling of headspace air. To avoid

215 underestimation of N_2 fixation rates due to poor diffusion, acetylene was added to the

216 incubation jars as acetylene-enriched seawater prepared according to Wilson et al. (2012),

217 thereby reducing equilibration time. Briefly, acetylene-enriched seawater was prepared by

218 bubbling acetylene gas (99.9%, supplied by Abdullah Hashim Industrial Gases &

219 Equipment Co. Ltd. Jeddah, Saudi Arabia) for five minutes. We added 20 ml of

220 acetylene-enriched seawater to each incubation jar through the gas-tight valve to obtain a

221 final acetylene concentration of 4 mM. After the addition of acetylene, samples were

222 incubated in Percival chambers for 24 h at the temperatures recorded *in situ* (35°C or

223 28°C) under light (200 μmol photons m^{-2} s^{-1}) and dark conditions following the natural

224 photoperiod (12 h light, 12 h dark). The following negative controls were run for each

225 sampling: 1) sediment samples without addition of acetylene-saturated seawater in order

226 to confirm that ethylene was not naturally produced; 2) seawater used in the preparation

227 of the sediment slurries with addition of acetylene-saturated seawater in order to measure

228 the N_2 fixation due to pelagic diazotrophs. During the 24 h incubations, we sampled the

229 headspace of each jar at five different time points by taking three ml of the headspace air.

230 The headspace air samples were injected in gas-tight vacuum vials using a syringe to be

231 analyzed later for ethylene production. The concentration of ethylene in the headspace air

232 samples was analyzed on a gas chromatographer coupled to a flame ionization detector

233 and a mass spectrometer (MS-FID-GC, Agilent 7890) using HP-AL/S (30 m $\times$ 0.250

234 mm $\times$ 5.0 μm) and GS-CarbonPLOT (60 m $\times$ 0.320 mm $\times$ 1.50 μm) columns (Agilent

235 Technologies, USA). We used two different columns due to the over use of the first

236 column. The ethylene concentration of each headspace air sample was calculated using a

237 standard curve, which was built from the peak area of three ethylene gas standards of

238 known concentrations (93 ppm, 9 ppm and 1.5 ppm containing Helium as balance gas

239 (Abdullah Hashim Industrial Gases & Equipment Co. Ltd. Jeddah, Saudi Arabia)). Three

240 replicates of each ethylene gas standard were analyzed to make the calibration curves for

241 each column and batch of samples. The concentration of dissolved ethylene before

242 equilibrium with the headspace was calculated from the ethylene concentration measured

243 in the equilibrated headspace samples according to Wilson et al. (2012) and applying the

244 solubility coefficient of ethylene extracted from Breitbarth et al. (2004) as a function of

245 temperature and salinity. Monthly mean *in situ* seawater salinity values measured at each

246 sampling time were used to calculate the solubility coefficient (i.e. 39.9 in Nov/Dec 2016

247 and 40.5 in Jan/Feb 2017). Acetylene reduction rates were converted to N_2 fixation rates

248 following the theoretical ratio 3:1; which means that one N_2 molecule is fixed for each

249 three acetylene molecules reduced (Alongi et al. 2000; 2002). No ethylene production

250 was detected in the negative controls.

251 **2.4. Statistical analysis**

252   Differences in sediment OM, organic C and N content, and isotopic ($\delta^{13}C$ and $\delta^{15}N$)

253   composition among mature mangrove sediments with high and low crab-density, and

254   juvenile mangrove sediments with high and low crab-density were tested by ANOVA

255   analysis followed by Tukey's post-hoc test. To evaluate the effect of the mangrove age,

256   temperature and crab density on sediment N_2 fixation rates, we performed an ANCOVA

257   by considering three explanatory variables: two categorical factors as Age (two levels,

258   mature and juvenile) and Temperature (two levels, 35°C and 28°C) both fixed and

259   orthogonal, and crab density as our continuous explanatory variable, since crab

260   abundance could not be tightly controlled and the replicated plots within different

261   treatments showed variability in crab densities (Fig. S1). To test our model, we performed

262   a generalized linear model with a Quasipoisson family of error. We updated the model to

263   the first significant terms using the function *update()* in R. Statistical analysis and graphs

264   were produced with JMP, PRISM, and R software (R core team, 2017) using "ggplot2"

265   package (Wickham 2010).

266   **3. Results**

267   **3.1. *In situ* temperature and salinity**

268   The air temperature at the study site ranged from 14°C in January to 40°C in July

269   during a one-year period from July 2016 to July 2017. Similarly, the surface sediment

270   temperature ranged annually from 11°C to 57°C, with maximum temperature recorded in

271   July and minimum temperature in February. Surface sediment temperature oscillated

272   from 11°C to 32°C in the colder period and from 30°C to 57°C in the hotter period.

273   Belowground sediment temperature ranged from approximately 15°C in February to

274   50°C in July. Belowground sediment temperature oscillated from 15°C to 29°C in the

275 colder period and from 30°C to 50°C in the hotter period. Seawater temperature was

276 35°C in November/December 2016 and 28°C in January/February 2017. Monthly mean

277 (± SEM) seawater salinity was 39.9 ± 0.01 from 13 November to 12 December 2016

278 (first sampling time when temperature was 35°C) and 40.5 ± 0.01 from 28 January to 27

279 February 2017 (second sampling time when temperature was 28°C).

280 **3.2. Sediment characteristics**

281 The sediment OM content differed among the four experimental treatments

282 (ANOVA, $F_{3, 16}$ = 196, p < 0.0001, Fig. S2A), with higher values (mean ± SEM) recorded

283 in mature mangrove sediments (3.26 ± 0.04 and 2.81 ± 0.04% of dry weight in high and

284 low crab-density sediments, respectively) compared to juvenile mangrove sediments

285 (2.06 ± 0.05 and 2.05 ±0.04% of dry weight in high and low crab-density sediments,

286 respectively). Similarly, the sediment organic C content differed among sediment types

287 (ANOVA, $F_{3, 16}$ = 72.14, p < 0.0001, Fig. S2B), with the highest organic C content

288 measured in mature mangrove sediments with high crab-density (0.38 ± 0.01 mmol C g

289 DW sed^{-1}), followed by mature mangrove sediments with low crab density (0.25 ± 0.01

290 mmol C g DW sed^{-1}) and juvenile mangrove sediments (0.21 ± 0.01 and 0.20 ± 0.01

291 mmol C g DW sed^{-1} in sediments supporting high and low crab-density, respectively).

292 The sediment N content also differed among sediment types (ANOVA, $F_{3, 16}$ = 6.89, p =

293 0.003, Fig. S2C). The N content in mature mangrove sediments supporting high crab-

294 density (0.04 ± 0.0003 mmol N g DW sed^{-1}) was significantly higher than the N content

295 in mature mangrove sediments supporting low crab-density (0.03 ± 0.002 mmol N g DW

296 sed^{-1}t), juvenile mangrove sediments supporting high crab density (0.03 ± 0.002 mmol N

297 g DW sed^{-1}) and juvenile mangrove sediments supporting low crab-density (0.03 ± 0.002

298   mmol N g DW sed^{-1}) sediments (Tukey post-hoc test, $p < 0.05$, $p < 0.001$ and $p < 0.001$,

299   respectively).

300   The sediment carbon isotopic (δ^{13}C) signature differed among sediment types

301   (ANOVA, $F_{3, 16} = 9.04$, $p = 0.001$, Fig. S2D). The sediment from mature mangrove with

302   low crab-density plots showed the heaviest average δ^{13}C (-19.20 ± 0.25 ‰) compared to

303   mature mangrove with high crab-density (-20.72 ± 0.09‰), juvenile mangrove with high

304   crab-density (-20.74 ± 0.34‰), and juvenile mangrove with low crab-density (-20.32 ±

305   0.20‰) sediments (Tukey post-hoc test, $p < 0.01$, $p < 0.01$ and $p < 0.05$, respectively).

306   The sediment N isotopic (δ^{15}N) signature ranged from 1.68 ± 0.24‰ and 1.76 ± 0.44‰ in

307   juvenile and mature mangrove sediments with low crab-density, to 2.16 ± 0.12‰ and 2.18

308   ± 0.86‰ in mature and juvenile mangrove sediments with high crab-density (Fig. S2E), but

309   these differences were not statistically significant (ANOVA, $F_{3, 16} = 0.27$, $p = 0.85$).

310   **3.3.   Sediment N$_2$ fixation rates**

311   Sediment N$_2$ fixation rates ranged 200-fold from 0.002 ± 0.002 mg N m^{-2} d^{-1} in

312   juvenile mangrove sediments with high crab-density at 35°C, to 0.46 ± 0.12 mg N m^{-2} d^{-1}

313   in mature mangrove sediments with low crab-density at 28°C (Fig. 1). The age of the

314   mangrove stand, temperature, and crab density had significant statistical effects on

315   sediment N$_2$ fixation rates, as did the interaction between temperature, crab density, and

316   age of the mangrove stand (GLM, Table 1). N$_2$ fixation rates were higher in mature

317   mangrove sediments than in juvenile sediments and the rates dropped when temperature

318   increased from 28°C to 35°C. Similarly, sediment N$_2$ fixation rates decreased under high

319   crab-density (Fig. 1), with a linear decrease in rates with increasing crab-density (Fig.

320   S3).

321   Sediment N_2 fixation rates measured in January/February at 28°C increased with

322   increasing sediment OM content in high crab-density (lm, $F_{1,8} = 7.63$, $p = 0.02$) and in

323   low crab-density (lm, $F_{1,8} = 12.17$, $p = 0.008$) mangrove sediments (Fig. 2A, B).

324   Similarly, sediment N_2 fixation rates increased with increasing sediment organic C

325   content in high (lm, $F_{1,8} = 7.68$, $p = 0.02$) and low crab-density (lm, $F_{1,8} = 12.45$, $p =$

326   0.008) mangrove sediments (Fig. 2C, D). However, the sediment N content and sediment

327   N_2 fixation rates at 28°C were not linearly related in high crab-density (lm, $F_{1,8} = 2.82$, p

328      $= 0.13$) and in low crab-density (lm, $F_{1,8} = 4.33$, $p = 0.07$) mangrove sediments (Fig. 2E,

329   F). Similarly, sediment N_2 fixation rates measured in January/February at 28°C increased

330   with heavier sediment C isotopic ($\delta^{13}C$) composition of organic matter pools (lm, $F_{1,18} =$

331   42.05, $p < 0.0001$, Fig. 3A). However, sediment N_2 fixation rates measured in

332   January/February at 28°C were not linearly related to the sediment N isotopic ($\delta^{15}N$)

333   composition (lm, $F_{1,18} = 3.3$, $p = 0.09$, Fig. 3B).

334   **4. Discussion**

335   This study provides evidence that high temperature and crab density can reduce N_2

336   fixation rates in mangrove sediments from arid regions. Sediment N_2 fixation rates

337   detected in *A. marina* mangrove stands in the Central Red Sea (from 0.0007 to 0.168 g N

338   m^{-2} yr^{-1}) were lower than the average N_2 fixation rates previously reported in mangroves

339   (from 0.03 to 2.6 g N m^{-2} yr^{-1}, Zuberer and Silver 1978; Howarth et al. 1988). The

340   comparatively low N_2 fixation rates measured here could be explained by the high salinity

341   values in our study site, in agreement with Vovides et al. (2011) that found a strong

342   negative relation between N_2 fixation rates and salinity in an impacted black mangrove in

343   the Gulf of Mexico. Central Red Sea mangrove stands are stunted, with low productivity

344 and with organic-poor sediments when compared to mangrove stands elsewhere

345 (Almahasheer, Duarte, and Irigoien 2016b; Almahasheer et al. 2017; Garcias-Bonet et al.

346 2019a), due to a critical nutrient limitation, including iron-limitation (Almahasheer,

347 Duarte, and Irigoien 2016a). The low N_2 fixation detected here is consistent with the iron-

348 limiting condition of these mangrove stands as the nitrogenase enzyme requires iron as a

349 cofactor (Howard and Rees 1996). Despite the low rates of sediment N_2 fixation, we

350 revealed a number of drivers eliciting a 200-fold range in N_2 fixation rates in central Red

351 Sea mangroves.

352 N_2 fixation was strongly temperature-dependent, with 10-fold higher rates at 28°C

353 than at 35°C, where higher temperatures resulted in undetectable N_2 fixation rates. This is

354 consistent with the thermal optima for N_2 fixation, ranging between 15°C and 30°C,

355 described in terrestrial ecosystems elsewhere (Houlton et al. 2008), and reports of reduced

356 N_2 fixation rates at > 30°C in desert soils (Barger, Castle, and Dean 2013; Zhou et al.

357 2016), and seagrass meadows (Garcias-Bonet et al. 2018; Garcias-Bonet et al. 2019b).

358 The temperature dependence of N_2 fixation can be explained by a thermal suppression of

359 nitrogenase synthesis (Brooks, Collins, and Brill 1984). In addition, temperature can

360 control the microbial community structure (Wang et al. 2013), including microorganisms

361 responsible for N_2 fixation. Although thermophilic N_2-fixing microorganisms have been

362 reported in anoxic environments and hydrothermal vents (Wahlund and Madigan 1993;

363 Mehta and Baross 2006), high temperature conditions clearly reduce N_2-fixing activity in

364 mangrove sediments in the Central Red Sea.

365 The age of mangrove trees, with the associated difference in stand and rhizosphere

366 development and biomass, had a significant effect on sediment N_2 fixation rates, with

367 higher rates in the mature mangrove stand than in the juvenile mangrove stand. This

368 suggests that mature mangrove stands, which also support increased sediment OM

369 content and organic C stocks, provide more suitable biogeochemical conditions favoring

370 N_2-fixing microbial communities (Holguin, Vazquez, and Bashan 2001; Inoue et al.

371 2019), as supported by the positive linear relationship of sediment OM and organic C

372 content and sediment N_2 fixation in both low and high crab-density plots. Sediment N_2

373 fixation was independent of sediment N content. However, since N_2 fixation is known to

374 be suppressed under high inorganic N concentrations (Knapp 2012), some of the

375 variability detected here could be also due to differences in ambient nitrate and

376 ammonium concentrations.

377   The difference in C isotopic ($\delta^{13}C$) composition of mangrove sediments, ranging

378 from -20.74 ± 0.34 to -19.20 ± 0.25‰, and mangrove tissues in the central Red Sea, -

379 26.08 ± 0.11‰ (average ± SEM, N=117, Almahasheer et al. 2017), suggests a potential

380 additional source other than mangrove tissues to their accumulated sediment OM.

381 Garcias-Bonet et al. (2019a) recently reported that mangrove leaves were the major

382 contributors (56 ± 8 %) to the accumulated sediment OM in Red Sea mangroves, with

383 additional, but minor, contributions from other primary producers, such as macroalgae,

384 seagrass, and halophytes, based on stable isotope mixing models. Similarly, the $\delta^{13}C$ of

385 mature low crab-density sediments (-19.20 ± 0.25‰) reported here likely indicates the

386 contribution of microphytobenthos, for which $\delta^{13}C$ has been reported between -14.4‰

387 and -13‰ (Oakes and Eyre 2014) and we recently measured $\delta^{13}C$ values of -15.07 ± 0.07

388 ‰ in microbial mats in Central Red Sea mangroves (Garcias-Bonet et al. 2019a). This

389 highlights the role of cyanobacteria as both a source of C and N, through N_2 fixation in

17

390   mature low crab-density mangrove sediments, holding the highest N_2 fixation rates.

391   However, N_2 fixation rates were independent of sediment N isotopic ($\delta^{15}N$) composition,

392   possibly indicating N inputs from additional sources to N_2 fixation (Kuramoto and

393   Minagawa 2001), as the rates measured were low, insufficient to satisfy the N demands of

394   the ecosystem.

395   Sediment N_2 fixation rates were negatively affected by crab density. Low crab-

396   density sediments consistently showed higher N_2 fixation rates than the high crab-density

397   sediments. Crab activity can affect N_2 fixation activity in several ways. First, crab

398   burrowing activity allows the transport of oxygen to deeper, anoxic sediment layers,

399   increasing oxygen concentration and redox potential in sediment around the burrow

400   (Booth et al. 2019) and making the conditions less favorable for N_2 fixation due to

401   potential inhibition of nitrogenase enzyme activity, which requires anaerobic conditions

402   (Goldberg, Nadler, and Hochman 1987; Reef, Feller, and Lovelock 2010). However,

403   burrowing activity could have a positive effect on mangrove growth by favoring

404   oxidation and release of iron from the sediment, which is limiting mangrove growth

405   (Mokhtari et al. 2016; Almahasheer, Duarte, and Irigoien 2016a). In addition, crabs can

406   limit N_2 fixation through their feeding behavior, which alters the microbial community in

407   the sediments. Cyanobacteria have an important role in N_2 fixation in mangrove

408   ecosystems (Alvarenga et al. 2015). Hence, crab grazing on cyanobacteria might reduce

409   N_2 fixation in the system, as supported by the differences in $\delta^{13}C$ values between low and

410   high crab-density in mature sediments, which suggest a somewhat lower contribution of

411   microbial mats to organic C pools in sediments affected by crab activity. We

412   acknowledge here that some crab species belonging to sesarmid family, which have a

413   herbivorous diet, can increase the C:N by storing organic matter such as leaves and

414   propagules in their burrows (Andreetta et al. 2014), and therefore creating the redox

415   conditions that favor the N_2 fixation (Booth et al. in press). However, this is not the case

416   in our study since central Red Sea mangroves are mainly colonized by fiddler and sand

417   bubble crabs with relatively few occurrences of sesarmid crabs, which are mainly located

418   in the south (Price et al. 1987). However, further research is needed to corroborate the

419   suggestion that the feeding behavior of crabs may contribute to reduce N_2 fixation, for

420   instance by microscopy or compound specific analysis.

421   Our experimental assessment does not capture ecosystem N_2 fixation, as we disturbed

422   the sediments to ensure a good distribution of acetylene, and some components of N_2

423   fixation in the mangrove system, such as activity associated with mangrove roots (Alfaro-

424   Espinoza and Ullrich 2015) or leaf litter (Pelegri, Rivera-Monroy, and Twilley 1998),

425   were not included as it would have biased the comparison between juvenile and mature

426   stands. Moreover, ARA has some methodological limitations to be considered, although it

427   has been extensively used to measure N_2 fixation rates in coastal vegetated sediments,

428   including mangrove (e.g. Romero et al. 2012), seagrass (e.g. Welsh 2000; Garcias-Bonet

429   et al. 2018; Garcias-Bonet et al. 2019b) and salt marsh (Murphy, Boyer, and Carpenter

430   2017) sediments. Specifically, addition of acetylene has been shown to affect the

431   microbial community composition (Fulweiler et al. 2015). Therefore, the N_2 fixation rates

432   reported here need to be carefully interpreted.

433   **5. Conclusions**

434   Sediment N_2 fixation rates in central Red Sea mangrove ecosystems are within the

435   low range among those reported for mangrove ecosystems, with almost undetectable N_2

436 fixation in sediments of juvenile mangrove stand. We identified temperature and crab

437 density as important interacting drivers of N_2 fixation, pointing out a complex regulation

438 of N_2 fixation in mangrove ecosystems. Rapid warming of the Red Sea (Chaidez et al.

439 2017) may, thus, further reduce N_2 fixation, thereby aggravating the nutrient-limiting

440 conditions of Red Sea mangroves.

441 **Author contributions**

442 NG-B, MF, DD and CMD designed this study. MF, JMB and DD design and set up the

443 experimental mangrove plots. MSQ, MF and NG-B performed the fieldwork. MSQ and

444 NG-B performed the atmospheric N_2 fixation measurements. NG-B, MF, MSQ and CMD

445 interpreted the results. MF, CMD and NG-B performed the statistical analysis. NG-B,

446 MF and MSQ wrote the first draft of the manuscript. All authors contributed substantially

447 to improving the manuscript.

454 **Declarations of interest:** none

455

References

Alfaro-Espinoza, Gabriela, and Matthias S. Ullrich. 2015. "Bacterial N2-Fixation in Mangrove Ecosystems: Insights from a Diazotroph-Mangrove Interaction." *Frontiers in Microbiology* 6: 445. https://doi.org/10.3389/fmicb.2015.00445.

Aller, Robert C. 1994. "Bioturbation and Remineralization of Sedimentary Organic Matter : Effects of Redox Oscillationl." *Chemical Geology* 114: 331–45.

Almahasheer, Hanan, Carlos M. Duarte, and Xabier Irigoien. 2016a. "Nutrient Limitation in Central Red Sea Mangroves." *Frontiers in Marine Science* 3 (December). https://doi.org/10.3389/fmars.2016.00271.

———. 2016b. "Phenology and Growth Dynamics of Avicennia Marina in the Central Red Sea." *Scientific Reports* 6 (1): 37785. https://doi.org/10.1038/srep37785.

Almahasheer, Hanan, Oscar Serrano, Carlos M. Duarte, Ariane Arias-Ortiz, Pere Masque, and Xabier Irigoien. 2017. "Low Carbon Sink Capacity of Red Sea Mangroves." *Scientific Reports* 7 (1). https://doi.org/10.1038/s41598-017-10424-9.

Alongi, D. M., F. Tirendi, L. A. Trott, and T. T. Xuan. 2000. "Benthic Decomposition Rates and Pathways in Plantations of the Mangrove Rhizophora Apiculata in the Mekong Delta, Vietnam." *Marine Ecology Progress Series* 194: 87–101. https://doi.org/10.3354/meps194087.

Alongi, D. M., L. A. Trott, G. Wattayakorn, and B. F. Clough. 2002. "Below-Ground Nitrogen Cycling in Relation to Net Canopy Production in Mangrove Forests of Southern Thailand." *Marine Biology* 140 (4): 855–64. https://doi.org/10.1007/s00227-001-0757-6.

Alvarenga, Danillo Oliveira, Janaina Rigonato, Luis Henrique Zanini Branco, and Marli Fátima Fiore. 2015. "Cyanobacteria in Mangrove Ecosystems." *Biodiversity and Conservation* 24 (4): 799–817. https://doi.org/10.1007/s10531-015-0871-2.

Andreetta, Anna, Marco Fusi, Irene Cameldi, Filippo Cimò, Stefano Carnicelli, and Stefano Cannicci. 2014. "Mangrove Carbon Sink. Do Burrowing Crabs Contribute to Sediment Carbon Storage? Evidence from a Kenyan Mangrove System." *Journal of Sea Research* 85 (January): 524–33. https://doi.org/10.1016/j.seares.2013.08.010.

Barger, Nichole N, Sarah C Castle, and Gavin N Dean. 2013. "Denitrification from Nitrogen-Fixing Biologically Crusted Soils in a Cool Desert Environment, Southeast Utah, USA." *Ecological Processes* 2 (1): 1. https://doi.org/10.1186/2192-1709-2-16.

Bertics, Victoria J., Jill A. Sohm, Tina Treude, Cheryl Emiliane T Chow, Douglas G. Capone, Jed A. Fuhrman, and Wiebke Ziebis. 2010a. "Burrowing Deeper into Benthic Nitrogen Cycling: The Impact of Bioturbation on Nitrogen Fixation Coupled to Sulfate Reduction." *Marine Ecology Progress Series* 409: 1–15. https://doi.org/10.3354/meps08639.

Bertics, Victoria J., Jill A. Sohm, Tina Treude, Cheryl-Emiliane T. Chow, Douglas G. Capone, Jed A. Fuhrman, and Wiebke Ziebis. 2010b. "Burrowing Deeper into Benthic Nitrogen Cycling: The Impact of Bioturbation on Nitrogen Fixation Coupled to Sulfate Reduction." *Marine Ecology Progress Series* 409 (June): 1–15. https://doi.org/10.3354/meps08639.

Booth, Jenny Marie, Marco Fusi, Ramona Marasco, Tumeka Mbobo, and Daniele
 Daffonchio. 2019. "Fiddler Crab Bioturbation Determines Consistent Changes in
 Bacterial Communities across Contrasting Environmental Conditions." *Scientific
 Reports* 9 (March). https://doi.org/10.1038/s41598-019-40315-0.

Boto, Kevin G., and Alistar I. Robertson. 1990. "The Relationship between Nitrogen
 Fixation and Tidal Exports of Nitrogen in a Tropical Mangrove System."
 Estuarine, Coastal and Shelf Science 31 (5): 531–40.
 https://doi.org/10.1016/0272-7714(90)90011-F.

Bouillon, Steven, Alberto V. Borges, Edward Castañeda-Moya, Karen Diele, Thorsten
 Dittmar, Norman C. Duke, Erik Kristensen, et al. 2008. "Mangrove Production
 and Carbon Sinks: A Revision of Global Budget Estimates." *Global
 Biogeochemical Cycles* 22 (2). https://doi.org/10.1029/2007GB003052.

Breitbarth, Eike, Matthew M. Mills, Gernot Friedrichs, and Julie LaRoche. 2004. "The
 Bunsen Gas Solubility Coefficient of Ethylene as a Function of Temperature and
 Salinity and Its Importance for Nitrogen Fixation Assays." *Limnology and
 Oceanography: Methods* 2 (8): 282–88. https://doi.org/10.4319/lom.2004.2.282.

Brooks, S J, J J Collins, and W J Brill. 1984. "Repression of Nitrogen-Fixation in
 Klebsiella Pneumoniae at High Temperature." *Journal of Bacteriology* 157 (2):
 460–64.

Capone, Douglas G. 1993. "Determination of Nitrogenase Activity in Aquatic Samples
 Using the Acetylene Reduction Procedure." In .

Chaidez, V., D. Dreano, S. Agusti, C. M. Duarte, and I. Hoteit. 2017. "Decadal Trends in
 Red Sea Maximum Surface Temperature." *Scientific Reports* 7 (1).
 https://doi.org/10.1038/s41598-017-08146-z.

Dean, Walter E. 1974. "Determination of Carbonate and Organic Matter in Calcareous
 Sediments and Sedimentary Rocks by Loss on Ignition; Comparison with Other
 Methods." *Journal of Sedimentary Research* 44 (1): 242–48.
 https://doi.org/10.1306/74D729D2-2B21-11D7-8648000102C1865D.

Donato, Daniel C., J. Boone Kauffman, Daniel Murdiyarso, Sofyan Kurnianto, Melanie
 Stidham, and Markku Kanninen. 2011. "Mangroves among the Most Carbon-Rich
 Forests in the Tropics." *Nature Geoscience* 4 (5): 293–97.
 https://doi.org/10.1038/ngeo1123.

Duarte, Carlos M., Antonio Delgado-Huertas, Andrea Anton, Paloma Carrillo-de-
 Albornoz, Daffne C. López-Sandoval, Susana Agustí, Hanan Almahasheer, et al.
 2018. "Stable Isotope (Δ13C, Δ15N, Δ18O, ΔD) Composition and Nutrient
 Concentration of Red Sea Primary Producers." *Frontiers in Marine Science* 5.
 https://doi.org/10.3389/fmars.2018.00298.

El-Juhany, Loutfy. 2009. "Present Status and Degradation Trends of Mangrove Forests
 on the Southern Red Sea Coast of Saudi Arabia." *J. Agric. Environ. Sci.* 6
 (January): 328–40.

Ferreira, T. O., X. L. Otero, P. Vidal-Torrado, and F. Macías. 2007. "Effects of
 Bioturbation by Root and Crab Activity on Iron and Sulfur Biogeochemistry in
 Mangrove Substrate." *Geoderma* 142 (1): 36–46.
 https://doi.org/10.1016/j.geoderma.2007.07.010.

Fulweiler, Robinson W., Elise M. Heiss, Mary Kate Rogener, Silvia E. Newell, Gary R.
 LeCleir, Sarah M. Kortebein, and Steven W. Wilhelm. 2015. "Examining the

Impact of Acetylene on N-Fixation and the Active Sediment Microbial Community." *Frontiers in Microbiology* 6: 418. https://doi.org/10.3389/fmicb.2015.00418.

Garcias-Bonet, Neus, Antonio Delgado-Huertas, Paloma Carrillo-de-Albornoz, Andrea Anton, Hanan Almahasheer, Nuria Marba, Iris Eline Hendriks, Dorte Krause-Jensen, and Carlos M. Duarte. 2019. "Carbon and Nitrogen Concentrations, Stocks, and Isotopic Compositions in Red Sea Seagrass and Mangrove Sediments." *Frontiers in Marine Science* 6. https://doi.org/10.3389/fmars.2019.00267.

Garcias-Bonet, Neus, Marco Fusi, Muhammad Ali, Dario R. Shaw, Pascal E. Saikaly, Daniele Daffonchio, and Carlos M. Duarte. 2018. "High Denitrification and Anaerobic Ammonium Oxidation Contributes to Net Nitrogen Loss in a Seagrass Ecosystem in the Central Red Sea." *Biogeosciences* 15 (23): 7333–46. https://doi.org/10.5194/bg-15-7333-2018.

Garcias-Bonet, Neus, Raquel Vaquer-Sunyer, Carlos M. Duarte, and Núria Marbà. 2019. "Warming Effect on Nitrogen Fixation in Mediterranean Macrophyte Sediments." *Biogeosciences* 16 (1): 167–75. https://doi.org/10.5194/bg-16-167-2019.

Giri, C., E. Ochieng, L. L. Tieszen, Z. Zhu, A. Singh, T. Loveland, J. Masek, and N. Duke. 2011. "Status and Distribution of Mangrove Forests of the World Using Earth Observation Satellite Data." *Global Ecology and Biogeography* 20 (1): 154–59.

Goldberg, I, V Nadler, and A Hochman. 1987. "Mechanism of Nitrogenase Switch-off by Oxygen." *Journal of Bacteriology* 169 (2): 874–79.

Holguin, Gina, Patricia Vazquez, and Yoav Bashan. 2001. "The Role of Sediment Microorganisms in the Productivity, Conservation, and Rehabilitation of Mangrove Ecosystems: An Overview." *Biology and Fertility of Soils* 33 (4): 265–78. https://doi.org/10.1007/s003740000319.

Houlton, Benjamin Z., Ying-Ping Wang, Peter M. Vitousek, and Christopher B. Field. 2008. "A Unifying Framework for Dinitrogen Fixation in the Terrestrial Biosphere." *Nature* 454 (7202): 327–30. https://doi.org/10.1038/nature07028.

Howard, James B, and Douglas C Rees. 1996. "Structural Basis of Biological Nitrogen Fixation." *Chemical Reviews* 96 (7): 2965–82. https://doi.org/10.1021/cr9500545.

Howarth, Robert W., Roxanne Marino, Judith Lane, and Jonathan J. Cole. 1988. "Nitrogen Fixation in Freshwater, Estuarine, and Marine Ecosystems. 1. Rates and Importance1." *Limnology and Oceanography* 33 (4part2): 669–87. https://doi.org/10.4319/lo.1988.33.4part2.0669.

Inoue, Tomomi, Ayako Shimono, Yasuaki Akaji, Shigeyuki Baba, Akio Takenaka, and Hung Tuck Chan. 2019. "Mangrove–Diazotroph Relationships at the Root, Tree and Forest Scales: Diazotrophic Communities Create High Soil Nitrogenase Activities in Rhizophora Stylosa Rhizospheres." *Annals of Botany*.

Jennerjahn, Tim C., and Venugopalan Ittekkot. 2002. "Relevance of Mangroves for the Production and Deposition of Organic Matter along Tropical Continental Margins." *Naturwissenschaften* 89 (1): 23–30. https://doi.org/10.1007/s00114-001-0283-x.

Kristensen, Erik. 2008. "Mangrove Crabs as Ecosystem Engineers; with Emphasis on Sediment Processes." *Journal of Sea Research* 59 (1–2): 30–43. https://doi.org/10.1016/j.seares.2007.05.004.

Kuramoto, Toshikatsu, and Masao Minagawa. 2001. "Stable Carbon and Nitrogen Isitopic Characterization of Organic Matter in a Mangrove Ecosystel on the Southwestern Coast of Thailand." *Journal of Oceanography*.

Laverock, Bonnie, Jack a Gilbert, Karen Tait, a Mark Osborn, and Steve Widdicombe. 2011. "Bioturbation: Impact on the Marine Nitrogen Cycle." *Biochemical Society Transactions* 39 (1): 315–20. https://doi.org/10.1042/BST0390315.

Lovelock, Catherine E., Alistair Grinham, Maria Fernanda Adame, and Helen M. Penrose. 2010. "Elemental Composition and Productivity of Cyanobacterial Mats in an Arid Zone Estuary in North Western Australia." *Wetlands Ecology and Management* 18 (1): 37–47. https://doi.org/10.1007/s11273-009-9146-6.

Luther, George W., Joel E. Kostka, Thomas M. Church, Barbara Sulzberger, and Werner Stumm. 1992. "Seasonal Iron Cycling in the Salt-Marsh Sedimentary Environment: The Importance of Ligand Complexes with Fe(II) and Fe(III) in the Dissolution of Fe(III) Minerals and Pyrite, Respectively." *Marine Chemistry* 40 (1–2): 81–103. https://doi.org/10.1016/0304-4203(92)90049-G.

Mandura, A. S. 1997. "A Mangrove Stand under Sewage Pollution Stress: Red Sea." *Mangroves and Salt Marshes* 1: 255–62. https://doi.org/10.1023/A:1009927605517.

Mazda, Yoshihiro, Michimasa Magi, Motohiko Kogo, and Phan Nguyen Hong. 1997. "Mangroves as a Coastal Protection from Waves in the Tong King Delta, Vietnam." *Mangroves and Salt Marshes* 1 (2): 127–35. https://doi.org/10.1023/A:1009928003700.

Mehta, Mausmi P., and John A. Baross. 2006. "Nitrogen Fixation at 92°C by a Hydrothermal Vent Archaeon." *Science* 314 (5806): 1783–86. https://doi.org/10.1126/science.1134772.

Mokhtari, Mohammad, Mazlan Abd Ghaffar, Gires Usup, and Zaidi Che Cob. 2016. "Effects of Fiddler Crab Burrows on Sediment Properties in the Mangrove Mudflats of Sungai Sepang, Malaysia." *Biology* 5 (1): 7. https://doi.org/10.3390/biology5010007.

Murphy, Jennifer Lynn, Katharyn Boyer, and Edward J. Carpenter. 2017. "Restoration of Cordgrass Salt Marshes: Limited Effects of Organic Matter Additions on Nitrogen Fixation." *Wetlands* 38: 361–71. https://doi.org/10.1007/s13157-017-0973-6.

Nagelkerken, I., S. J.M. Blaber, S. Bouillon, P. Green, M. Haywood, L. G. Kirton, J. O. Meynecke, et al. 2008. "The Habitat Function of Mangroves for Terrestrial and Marine Fauna: A Review." *Aquatic Botany* 89 (2): 155–85. https://doi.org/10.1016/j.aquabot.2007.12.007.

Oakes, J. M., and B. D. Eyre. 2014. "Transformation and Fate of Microphytobenthos Carbon in Subtropical, Intertidal Sediments: Potential for Long-Term Carbon Retention Revealed by ^{13}C-Labeling." *Biogeosciences* 11 (7): 1927–40. https://doi.org/10.5194/bg-11-1927-2014.

Pelegri, SP, VH Rivera-Monroy, and Robert Twilley. 1998. "A Comparison of Nitrogen Fixation (Acetylene Reduction) among Three Species of Mangrove Litter,

637   Sediments, and Pneumatophores in South Florida, USA (Vol 356, Pg 73, 1997)."

638   *Hydrobiologia* 361 (January): 239–40.

639 Polidoro, Beth A., Kent E. Carpenter, Lorna Collins, Norman C. Duke, Aaron M. Ellison,

640   Joanna C. Ellison, Elizabeth J. Farnsworth, et al. 2010. "The Loss of Species:

641   Mangrove Extinction Risk and Geographic Areas of Global Concern." Edited by

642   Dennis Marinus Hansen. *PLoS ONE* 5 (4).

643   https://doi.org/10.1371/journal.pone.0010095.

644 Price, A, P A. H. Medley, R J. McDowall, A R. Dawson-Shepherd, Peter Hogarth, and

645   Rupert Ormond. 1987. "Aspects of Mangal Ecology along the Red Sea Coast of

646   Saudi Arabia." *Journal of Natural History - J NATUR HIST* 21 (April): 449–64.

647   https://doi.org/10.1080/00222938700771121.

648 Ray, Raghab, Natasha Majumder, Subhajit Das, Chumki Chowdhury, and Tapan Kumar

649   Jana. 2014. "Biogeochemical Cycle of Nitrogen in a Tropical Mangrove

650   Ecosystem, East Coast of India." *Marine Chemistry* 167: 33–43.

651   https://doi.org/10.1016/j.marchem.2014.04.007.

652 Reef, Ruth, Ilka C. Feller, and Catherine E. Lovelock. 2010. "Nutrition of Mangroves."

653   *Tree Physiology* 30 (9): 1148–60. https://doi.org/10.1093/treephys/tpq048.

654 Reinsel, K.A. 2004. "Impact of Fiddler Crab Foraging and Tidal Inundation on an

655   Intertidal Sandflat: Season-Dependent Effects in One Tidal Cycle." *Journal of*

656   *Experimental Marine Biology and Ecology* 313 (1): 1–17.

657   https://doi.org/10.1016/j.jembe.2004.06.003.

658 Romero, Isabel C., Myrna Jacobson, Jed A. Fuhrman, Marilyn Fogel, and Douglas G.

659   Capone. 2012. "Long-Term Nitrogen and Phosphorus Fertilization Effects on N2

660   Fixation Rates and NifH Gene Community Patterns in Mangrove Sediments."

661   *Marine Ecology* 33 (1): 117–27. https://doi.org/10.1111/j.1439-

662   0485.2011.00465.x.

663 Saifullah, S. M. 1997. "Mangrove Ecosystem of Red Sea Coast (Saudi Arabia)." *Pakistan*

664   *Journal of Marine Sciences* 6 (1 & 2): 115–24.

665 Simmons, T. Luke, R. Cameron Coates, Benjamin R. Clark, Niclas Engene, David

666   Gonzalez, Eduardo Esquenazi, Pieter C. Dorrestein, and William H. Gerwick.

667   2008. "Biosynthetic Origin of Natural Products Isolated from Marine

668   Microorganism-Invertebrate Assemblages." *Proceedings of the National Academy*

669   *of Sciences of the United States of America* 105 (12): 4587–94.

670   https://doi.org/10.1073/pnas.0709851105.

671 Skov, M., M. Vannini, J. Shunula, R. Hartnoll, and S. Cannicci. 2002. "Quantifying the

672   Density of Mangrove Crabs: Ocypodidae and Grapsidae." *Marine Biology* 141

673   (4): 725–32. https://doi.org/10.1007/s00227-002-0867-9.

674 Wahlund, Thomas M, and Michael T Madigan. 1993. "Nitrogen Fixation by the

675   Thermophilic Green Sulfur Bacterium Chlorobium Tepidum" 175 (2): 474–78.

676 Walters, Bradley B., Patrik Rönnbäck, John M. Kovacs, Beatrice Crona, Syed Ainul

677   Hussain, Ruchi Badola, Jurgenne H. Primavera, Edward Barbier, and Farid

678   Dahdouh-Guebas. 2008. "Ethnobiology, Socio-Economics and Management of

679   Mangrove Forests: A Review." *Aquatic Botany*, Mangrove Ecology –

680   Applications in Forestry and Costal Zone Management, 89 (2): 220–36.

681   https://doi.org/10.1016/j.aquabot.2008.02.009.

682 Wang, Shang, Weiguo Hou, Hailiang Dong, Hongchen Jiang, Liuqin Huang, Geng Wu,
683   Chuanlun Zhang, et al. 2013. "Control of Temperature on Microbial Community
684   Structure in Hot Springs of the Tibetan Plateau." Edited by Josh Neufeld. *PLoS*
685   *ONE* 8 (5). https://doi.org/10.1371/journal.pone.0062901.
686 Welsh, D. T. 2000. "Nitrogen Fixation in Seagrass Meadows: Regulation, Plant–Bacteria
687   Interactions and Significance to Primary Productivity." *Ecology Letters* 3 (1): 58–
688   71. https://doi.org/10.1046/j.1461-0248.2000.00111.x.
689 Wickham, Hadley. 2010. "A Layered Grammar of Graphics." *Journal of Computational*
690   *and Graphical Statistics* 19 (1): 3–28. https://doi.org/10.1198/jcgs.2009.07098.
691 Wilson, Samuel T., Daniela Böttjer, Matthew J. Church, and David M. Karl. 2012.
692   "Comparative Assessment of Nitrogen Fixation Methodologies, Conducted in the
693   Oligotrophic North Pacific Ocean." *Applied and Environmental Microbiology* 78
694   (18): 6516–23. https://doi.org/10.1128/AEM.01146-12.
695 Zhou, Xiaobing, Hilda Smith, Ana Giraldo Silva, Jayne Belnap, and Ferran Garcia-
696   Pichel. 2016. "Differential Responses of Dinitrogen Fixation, Diazotrophic
697   Cyanobacteria and Ammonia Oxidation Reveal a Potential Warming-Induced
698   Imbalance of the N-Cycle in Biological Soil Crusts." *PLoS ONE* 11 (10): 1–15.
699   https://doi.org/10.1371/journal.pone.0164932.
700 Zuberer, D A, and W S Silver. 1978. "Biological Dinitrogen Fixation (Acetylene
701   Reduction) Associated with Florida Mangroves," no. 3: 567–75.
702

703   **Table 1**. Generalized linear model adopted to assess the effect of each factor and their

704   interaction on sediment N_2 fixation rates. Df: degree of freedom, Rs.Df: residual degree

705   of freedom, Rs.Dev: residual deviance; p: p statistic. The statistically significant results

706   are shown in bold.

Terms	Df	Deviance	Rs.Df.	Rs.Dev	p
Temperature	1	1.75094	38	6.1633	**< 0.0001**
Age	1	3.10967	37	3.0537	**< 0.0001**
Crab	1	2.10009	36	0.9536	**< 0.0001**
Temperature × Age	1	0.04009	35	0.9135	0.162441
Temperature × Crab	1	0.09976	34	0.8137	**0.027556**
Age × Crab	1	0.20814	33	0.6056	**0.01458**

707
708

Figures

710 **Fig. 1.** N_2 fixation rates in juvenile (a, b) and mature (c, d) mangrove sediments with high

711 crab-density (black bars) and low crab-density (gray bars) at 28°C and 35°C.
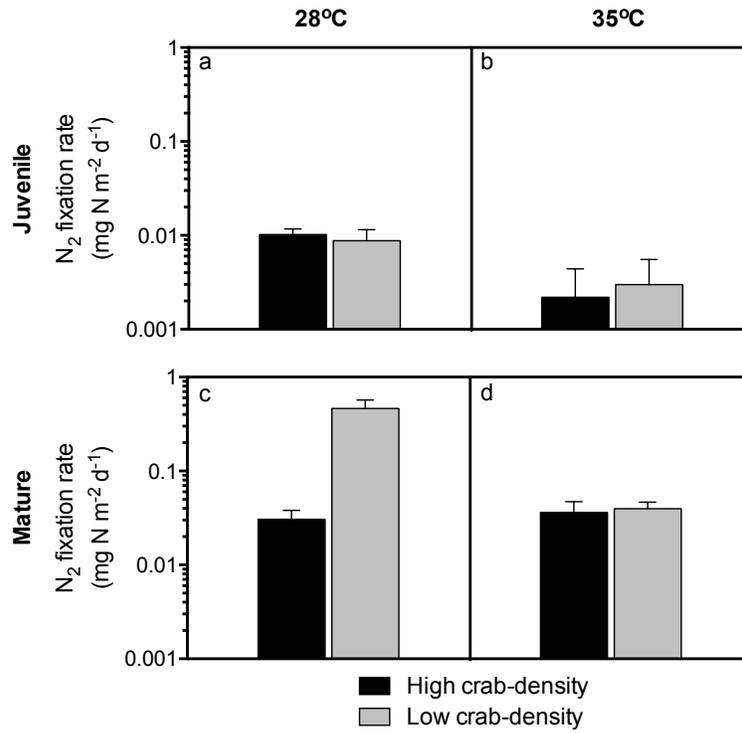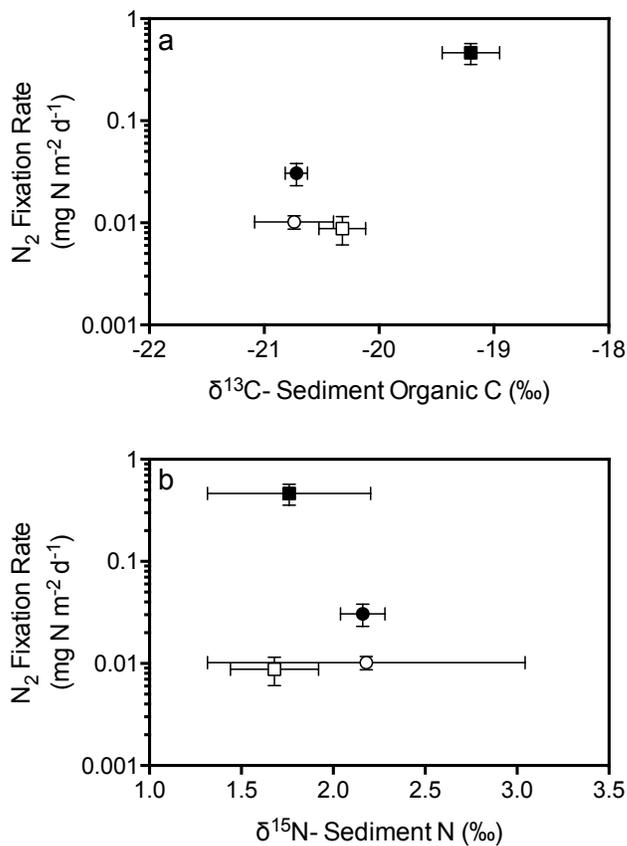

712

713

714

715 **Fig. 2.** Relation of N$_2$ fixation rates at 28°C in mangrove sediments with high and low-

716 crab density with sediment organic matter (a, b), organic carbon (c, d) and nitrogen (e, f)

717 content, showing linear regressions (dashed line). N$_2$ fixation rates in mature mangrove

718 sediments are represented as black dots and in juvenile mangrove sediments are

719 represented as white dots.
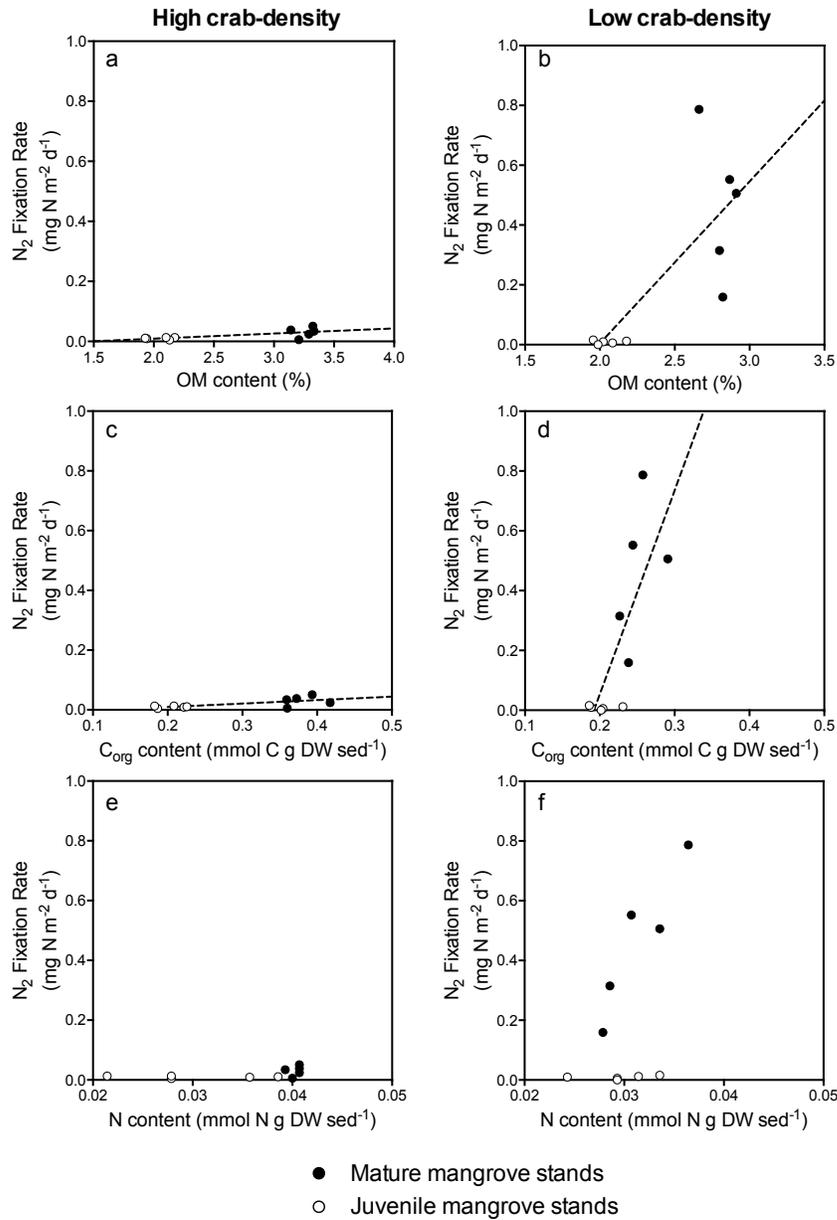
720

721 **Fig. 3.** Relation of sediment N_2 fixation rates at 28°C with sediment C isotopic ($\delta^{13}C$)

722 signature (a) and sediment N isotopic ($\delta^{15}N$) signature (b). Mature mangrove sediments

723 with high-crab density are represented as black dots, mature mangrove sediments with

724 low-crab density are represented as black squares, juvenile mangrove sediments with

725 high-crab density are represented as white dots, and juvenile mangrove sediments with

726 low-crab density are represented as white squares. Error bars indicate SEM.


727

728

729

730 **Supplementary figures**

731 **Figure S1.** Box plot showing crab density in high crab-density (High) and low crab-

732 density (Low) plots in juvenile and mature mangrove stands at two different sampling

733 times: in November/December 2016 when seawater temperature was 35°C and in

734 January/February 2017 when seawater temperature was 28°C. Boxes extend from the 25th

735 to 75th percentiles and lines inside boxes represent median values.

736
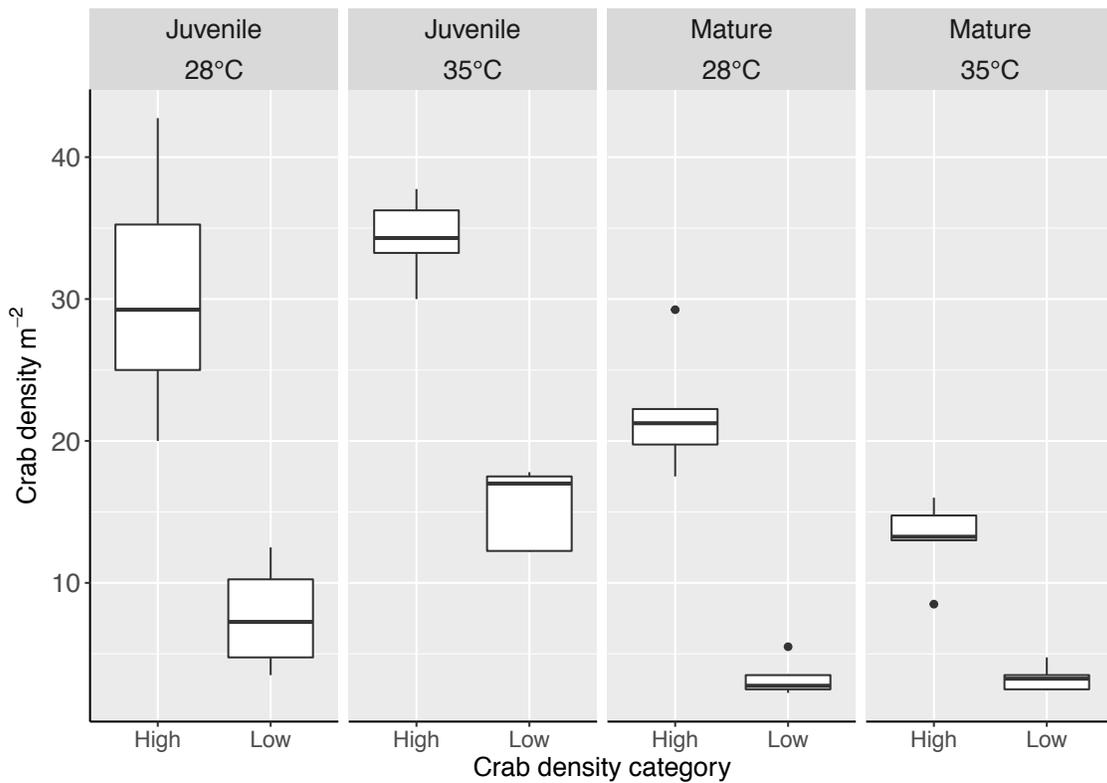
743 **Figure S2**. Sediment OM content (a), organic C content (b), N content (c), isotopic C

744 composition (d) and isotopic N composition (e) in mature with high-crab density (M

745 High), mature with low crab-density (M Low), juvenile with high crab-density (J High)

746 and juvenile low crab density (J Low) mangrove sediments in January/February 2017

747 when seawater temperature was 28°C. Boxes extend from the 25th to 75th percentiles,

748 lines inside boxes represent median values, and whiskers span from minimum to

749 maximum values. Letters indicate significant differences among groups based on

750 ANOVA analysis and Tuckey's post-hoc tests.
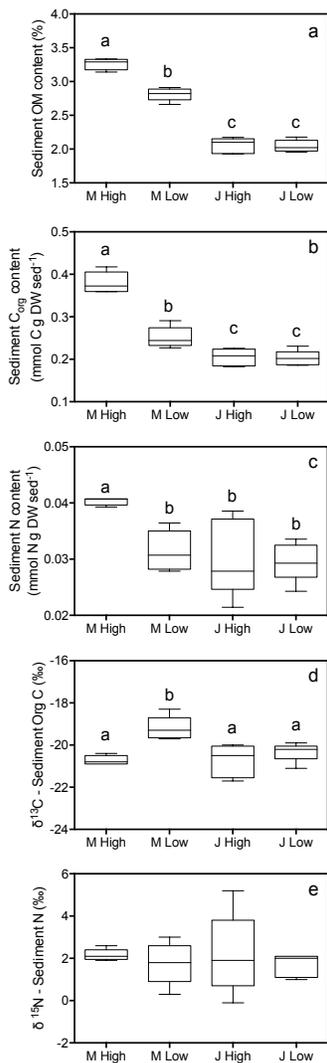
751

752 **Figure S3.** N_2 fixation rates in juvenile (upper panels) and mature (bottom panels)

753 mangrove sediments versus crab density at 28°C and 35°C. Black line is the regression

754 obtained with the function *geom_smooth()* of the package *ggplot2* in R and the gray area

755 is the 95% interval of confidence.
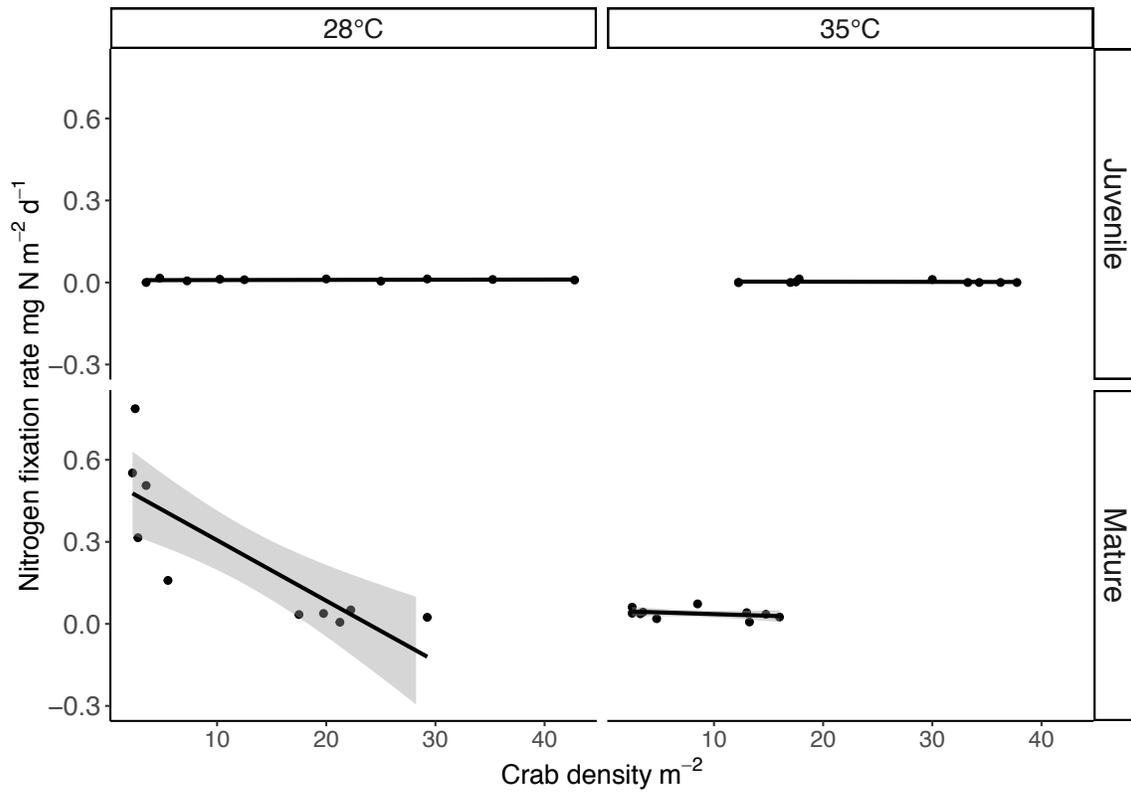
756

757

758

759 **Supplementary Table**

760 Raw data available in Supplementary Table S1

761