High denitrification and anaerobic ammonium oxidation contributes to net nitrogen loss in a seagrass ecosystem in the central Red Sea

Garcias-Bonet, Neus[1]*; Fusi, Marco[1]; Ali, Muhammad[2]; Shaw, Dario R.[2]; Saikaly, Pascal E.[2]; Daffonchio, Daniele[1] and Duarte, Carlos M.[1]

[1] King Abdullah University of Science and Technology, Red Sea Research Center, Thuwal 23955-6900, Saudi Arabia

[2] King Abdullah University of Science and Technology, Water Desalination and Reuse Center, Thuwal 23955-6900, Saudi Arabia

***corresponding author**: Garcias-Bonet, Neus.

Red Sea Research Center, Division of Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology (KAUST), Thuwal, 23955-6900, Kingdom of Saudi Arabia. Phone: +966 (012) 8082564.

E-mail: neus.garciasbonet@kaust.edu.sa

Running head: Nitrogen removal by seagrasses

Key words: Seagrass ecosystems, Coastal sediments, Atmospheric dinitrogen fixation, Denitrification, Anaerobic ammonium oxidation (Anammox), Sediment microprofiles, Red Sea

Abstract

Nitrogen loads in coastal areas have increased dramatically with detrimental consequences for coastal ecosystems. Shallow sediments and seagrass meadows are hotspots for denitrification, favoring N loss. However, atmospheric dinitrogen (N_2) fixation has been reported to support seagrass growth. Therefore, the role of coastal marine systems dominated by seagrasses in the net N_2 flux remains unclear. Here, we measured denitrification, anaerobic ammonium oxidation (anammox), and N_2 fixation in tropical seagrass (*Enhalus acoroides*) meadow and the adjacent bare sediment in a coastal lagoon in the central Red Sea. We detected high annual mean rates of denitrification (34.9 ± 10.3 and 31.6 ± 8.9 mg N m^{-2} d^{-1}) and anammox (12.4 ± 3.4 and 19.8 ± 4.4 mg N m^{-2} d^{-1}) in vegetated and bare sediments. The annual mean N loss was higher (8 and 63-fold higher) than the N_2 fixed (annual mean=5.9 ± 0.2 and 0.8 ± 0.3 mg N m^{-2} d^{-1}) in the meadow and bare sediment, leading to a net flux of N_2 from sediments to the atmosphere. Despite the importance of this coastal lagoon in removing N from the system, N_2 fixation can contribute substantially to seagrass growth since N_2 fixation rates found here could contribute up to 36% of plant N requirements. In vegetated sediments, anammox rates decreased with increasing organic matter (OM) content, while N_2 fixation increased with OM content. Denitrification and anammox increased linearly with temperature, while N_2 fixation showed a maximum at intermediate temperatures. Therefore, the forecasted warming could further increase the N_2 flux from sediments to the atmosphere, potentially impacting seagrass productivity and their capacity to mitigate climate change but also enhancing their potential N removal.

1. Introduction

Nutrient supply is an important driver of marine primary production (Field et al., 1998;Howarth, 1988), where nitrogen (N) availability is believed to exert a key role in regulating net primary production (Howarth, 1988) and driving eutrophication (Howarth and Marino, 2006) in coastal ecosystems. Anthropogenic activities have led to a doubling of the global amount of fixed N, with important changes in ecosystem productivity, diversity, air quality, and, ultimately, climate (Fowler et al., 2013;Vitousek et al., 1997). Whereas natural atmospheric dinitrogen (N_2) fixation is globally estimated at 203 Tg N yr^{-1} (from which 140 Tg N yr^{-1} occurs in marine systems), the anthropogenic contribution to new N supply has been estimated at 210 Tg N yr^{-1}, mainly produced by N_2-fixing crops, combustion of fossil fuels and the Haber-Bosch industrial reaction (Fowler et al., 2013).

Coastal areas receive high inputs of fixed N by river and groundwater discharges and atmospheric deposition (Galloway et al., 2003;Voss et al., 2013), causing severe problems related to eutrophication and, potentially, dystrophic crisis (Galloway et al., 2003;Herbert, 1999). High N inputs can be partially balanced through losses, as coastal marine sediments are hotspots of denitrification (Devol, 2015), the conversion of nitrates and nitrites to N_2 (and N_2O partially), leading to the loss of fixed N. Globally, coastal denitrification has been estimated to range from 4 to 8 Tg N yr^{-1} (Voss et al., 2013), which is modest compared to the global riverine input of 66 Tg N yr^{-1} (Seitzinger et al., 2005), N_2 fixation of about 15 Tg N yr^{-1} (Voss et al., 2013), and atmospheric deposition of 1 Tg N yr^{-1} (Voss et al., 2013) to the coastal ocean. Recently, however, anaerobic ammonium oxidation (anammox), the chemoautotrophic conversion of ammonium and nitrite to N_2, has also been regarded as an important process in marine

65 sediments. It has been estimated to account from nearly zero to up to 80% of the total N loss in
66 sediments (Dalsgaard et al., 2005;Devol, 2015).
67 Within the coastal ocean, seagrass ecosystems support high rates of N_2 fixation (McGlathery,
68 2008), particularly so in tropical and subtropical ecosystems (Welsh, 2000;Herbert, 1999).
69 Nitrogen supplied by N_2 fixation can account for up to 90% of plant nutrient requirements
70 (Hansen et al., 2000). It has been suggested that seagrasses have a close mutualistic relationship
71 with N_2-fixing bacteria inhabiting the rhizosphere (Herbert, 1999) and roots (Garcias-Bonet et
72 al., 2016), where bacteria would benefit from root exudates and plants would benefit from fixed
73 N supply. Yet, seagrass ecosystems also support high denitrification rates (Eyre et al., 2011b)
74 and have been identified as key habitats for N loss in temperate (Eyre et al., 2016) and sub-
75 tropical (Eyre et al., 2011a) estuaries. Although anammox rates have not been assessed for
76 seagrass sediments as yet, Salk et al. (2017) recently reported very low anammox rates (0.18
77 μmol N m^{-2} h^{-1}), although accounting for 74% of N loss, on bare sediment adjacent to a *Zostera*
78 *muelleri* meadow in a sub-tropical estuarine system. Therefore, the role of seagrass ecosystems
79 as net sinks or sources of N remains unclear. Welsh et al. (2000) reported very low
80 denitrification rates compared to N_2 fixation rates in a temperate intertidal seagrass meadow,
81 whereas denitrification seems to exceed N_2 fixation in tropical (Alongi et al., 2008) and sub-
82 tropical (Eyre et al., 2011a) seagrass ecosystems. The balance between transformations of inert
83 atmospheric N_2 and reactive N in seagrass ecosystems is an important driver of their net N
84 budget (Hemminga et al., 1991), particularly so in areas with limited N inputs from land. This is
85 the case of seagrass meadows in arid regions lacking riverine inputs, such as the Red Sea,
86 characterized by general oligotrophic conditions in surface waters (Raitsos et al., 2013;Weikert,
87 1987).

88 Nitrogen cycling in seagrass ecosystems is mediated by complex microbial communities
89 requiring specific physicochemical conditions, which may ultimately determine the balance
90 between transformations of inert atmospheric N_2 and reactive N. Seagrass meadows offer patchy
91 sediment conditions, affected by the release of organic exudates and oxygen by seagrass roots
92 (Pedersen et al., 1998), as well as the activity of burrowing infauna, which facilitates the
93 exchange and diffusion of nutrients and allows the oxygenation of deep sediment layers (Welsh,
94 2003). These processes contribute to high spatial heterogeneity in seagrass sediments, therefore,
95 modifying the redox potential (Enriquez et al., 2001) and allowing for the co-occurrence of
96 processes requiring different environmental conditions (Herbert, 1999;Hemminga et al., 1991).
97 Here, we test the following hypotheses: i) that seagrasses and bare sediments in a coastal lagoon
98 in the Red Sea are net N_2 sources and ii) that the loss of reactive N from sediments to the
99 atmosphere increases with temperature. Specifically, we assess the annual balance between
100 losses of reactive N as N_2, via denitrification and anammox, and gains of reactive N, by N_2
101 fixation, in a tropical seagrass (*Enhalus acoroides*) meadow and the adjacent bare sediment in a
102 coastal lagoon located in the central Red Sea. We first describe the environmental conditions in
103 the sediments, based on microprofiles of oxygen (O_2), sulfide (H_2S), and redox, and then
104 evaluate denitrification, anammox, and N_2 fixation rates in seagrass sediments and adjacent bare
105 sediments. In addition, we analyze the thermal dependence of denitrification, anammox, and N_2
106 fixation throughout the annual *in situ* thermal range. The Red Sea is one of the warmest seas and
107 is warming faster than other seas (Chaidez et al., 2017), thereby offering an opportunity to assess
108 if the balance between losses of reactive N as N_2, via denitrification and anammox, and gains of
109 reactive N, by N_2 fixation, may be affected by warming.
110

2. Materials and methods

2.1. Study site

The study was conducted on an *Enhalus acoroides* seagrass meadow in Khor Almesena'a, a shallow enclosed coastal lagoon located in the central Red Sea (22°23'23.2" N, 39°08'08" E). The study site was on a monospecific seagrass meadow at 2-m depth and the adjacent (about 5 m from the edge of the seagrass meadow) bare sediment. The study was conducted between June 2016 and April 2017. We assessed sediment microprofiles (O_2, H_2S, and redox) and denitrification, anammox, and N_2 fixation rates along five sampling events, two in summer (June and August 2016), one in the fall (November 2016), one in winter (February 2017), and one in spring (April 2017). At each sampling time, we also monitored and recorded salinity and seawater temperature *in situ* every 10 min for 24 hours using an EXO1 Multiparameter Sonde (YSI, USA).

2.2. Sediment microprofiles

At each sampling event, we performed O_2, H_2S, and redox microprofiles on vegetated sediments and adjacent bare sediment reaching an average depth of 7 cm below the sediment surface, using the Field Microprofiling system by Unisense (Aarhus, Denmark). At each sampling event, we collected four sediment cores (40 cm length and 10 cm in diameter, two replicate cores per each sediment type) containing at least 15 cm of undisturbed sediment. The cores were transported immediately to the laboratory and the microprofile analysis started within the next 3 h. Oxygen microsensors (Ox-200), with a tip diameter of 200 µm, were calibrated in sterile water at oxygen partial pressures of 0 and 21 kPa. For H_2S measurements, we used H_2S-200 microelectrodes (200 µm tip diameter). Calibration of the microsensors was performed following manufacturer

specifications and following Seitaj et al. (Seitaj et al., 2015). Redox measurements were performed using Redox-200 microelectrodes (200 μm tip diameter). The electrode potentials in the sediment were measured against the Unisense Ag-AgCl reference electrode and the calibration was performed using two quinidrone solutions (10 mg ml^{-1}) buffered at pH 4 and 7, respectively. Microsensors were positioned using a manual micromanipulator (Märzhäuser, Wetzlar, Germany), and the tip position was visually controlled with a horizontally mounted USB stereomicroscope (Veho VMS-004). Oxygen, H_2S, and redox microprofiling measurements started 10 min after embedding and lasted about 4 hours per sediment core. The sediment surface position was adjusted using the software Sensor Trace Suite v2.7.100 (Unisense, Aarhus, Denmark). The microprofiles were performed under light and dark conditions per triplicate. The cores were left for 12 h under dark conditions before starting the microprofiles under dark conditions.

2.3. Denitrification and anammox rates

We measured denitrification and anammox rates by a batch incubation experiment with ^{15}N-labeled nitrogen compounds in vegetated sediment and the adjacent bare sediment. At each sampling event, we collected 6 cylindrical plastic cores (40 cm length and 5 cm in diameter, 3 replicate cores per each sediment type) containing at least 15 cm of undisturbed sediment. The cores were transported immediately to the laboratory. Denitrification and anammox rates were measured at three sediment horizons: from sediment surface to 1 cm deep, from 1 to 2 cm deep and from 2 to 3 cm deep. ^{15}N isotope pairing technique was applied for measurement of N-related activities. The principle and procedure for measuring N_2 production via anammox were essentially based on a ^{15}N-tracer incubation method reported elsewhere (Thamdrup and

157 Dalsgaard, 2002). However, in this study, we used a simpler method developed previously
158 (Amano et al., 2011;Yoshinaga et al., 2011). Briefly, about two grams of sediments was
159 dispensed into 10-ml glass serum vials, which were sealed with butyl rubber stoppers and
160 aluminum caps. All these procedures were performed in an anaerobic chamber (Coy Laboratory
161 Products, Grass Lake Charter Township, MI) where O_2 concentration was <1 ppm. Headspace in
162 the vials was exchanged with highly pure He gas (>99.9999%) by vacuuming and purging.
163 Positive pressure (50-75 kPa) was added to the headspace to prevent unintentional contamination
164 with ambient air during the incubation and gas sampling. The vials containing oxygen-free
165 sediment suspensions were pre-incubated overnight in the dark to eliminate the remaining
166 substrates. Four different combinations of ^{15}N labeled and/or unlabeled substrates were
167 supplemented from anoxic stock solutions to these pre-incubated vials: 1) 0.5 mM $^{15}NH_4Cl$ (≥98
168 atom % ^{15}N, Sigma-Aldrich, Inc.); 2) 0.5 mM $^{15}NH_4Cl$ and 0.5 mM $Na^{14}NO_2$ (Sigma-Aldrich,
169 Inc.); 3) 0.5 mM $Na^{15}NO_2$ (98 atom % ^{15}N, Sigma-Aldrich, Inc.); and 4) 0.5 mM $K^{15}NO_3$ (98
170 atom % ^{15}N, Sigma-Aldrich, Inc.). The concentration of the ^{15}N-labeled and unlabeled substrate
171 was chosen as suggested previously (Amano et al., 2007). The vials were incubated under anoxic
172 conditions at *in situ* temperature. The concentrations of $^{29}N_2$ and $^{30}N_2$ gas were determined by
173 gas chromatography-mass spectrometry (GC-MS) analysis at different intervals as described
174 previously (Isobe et al., 2011a;Isobe et al., 2011b). In combination with GC inlet and peak
175 integration software, a quadrupole MS system can be used to determine N_2 production rates < 10
176 nmol N l^{-1} d^{-1} (Holtappels et al., 2011). Fifty microliters of headspace gas were collected using a
177 gas-tight syringe (VICI; Baton Rouge, LA, USA), immediately injected into a GC (Agilent
178 7890A system equipped with a CP-7348 PoraBond Q column) combined with 5975C quadrupole
179 inert MS (Agilent Technologies; Santa Clara, CA, USA). The m/z = 29 and 30 values monitored

180 at the same retention time for each measurement. The amounts of $^{29}N_2$ and $^{30}N_2$ gas were
181 determined using a standard curve prepared with $^{30}N_2$ standard gas (> 98% purity) (Cambridge
182 Isotope Laboratories; Tewksbury, MA, USA). The potential denitrification and anammox rates
183 were estimated from the production of $^{29}N_2$ and $^{30}N_2$ using the equations (provided in
184 Supplementary Materials) described previously (Holtappels et al., 2011;Yoshinaga et al., 2011).
185 All the batch tests were performed in triplicate. Finally, the denitrification and anammox rates
186 were standardized to surface area integrating 3 cm sediment depth by averaging the rates
187 measured at different horizons and taking into account the sediment bulk density.
188
189 **2.4. Atmospheric N_2 fixation rates**
190 We measured N_2 fixation by Acetylene Reduction Assay (Capone and Taylor, 1980) in seagrass
191 roots, seagrass shoot epiphytes, vegetated sediment, and adjacent bare sediment. At each
192 sampling time, we carefully collected shoots containing roots and placed them in zip-lock plastic
193 bags. The shoots were immediately transported to the laboratory in a cooler box protected from
194 sunlight. We also collected six cylindrical plastic cores (40 cm length and 10 cm in diameter,
195 three replicate cores per each sediment type) containing at least 15 cm of undisturbed sediment.
196 The sediment cores were transported immediately to the laboratory. Once in the laboratory the
197 roots were carefully separated from shoots and rinsed with sterilized seawater. Then, we placed
198 10 g (fresh weight) of healthy roots in a 250 ml glass bottle. Similarly, we placed one shoot
199 (without roots) in a 500 ml glass bottle. We added 100 and 250 ml of fresh seawater collected
200 from the same location to the root and shoot samples, respectively, and the bottles were closed
201 with lids fitted with gas-tight valves. In parallel, the sediment from the cores was extruded
202 carefully using a plunger, and the sediment was cut in four different horizons: from sediment

surface to 1 cm deep, from 1 to 2 cm deep, from 2 to 3 cm deep, and from 9 to 10 cm deep. We added the additional deeper sediment layer (9 to 10 cm), matching the maximum depth at which seagrass roots were detected. For each horizon, 80 ml of sediment was placed in a 500 ml glass bottle. Then, we added 200 ml of fresh seawater collected from the same location and the bottles were closed with a lid fitted with a gas-tight valve. Finally, we added acetylene-saturated seawater, prepared according to Wilson et al. (2012), through the gas-tight valve in order to achieve a final acetylene concentration of 4 mM (10 ml to roots and 20 ml to shoots and sediment incubations). The acetylene was added in the form of acetylene-saturated seawater to reduce the acetylene equilibration time and, therefore, avoid potential underestimation of ethylene production rates (Wilson et al., 2012). We ran the root and shoot incubations in triplicate. Similarly, we run the sediment incubation in triplicate for each horizon and sediment type. The roots and sediment slurries were incubated under dark conditions, and the shoots were incubated mimicking the natural photoperiod (12 h light at 200 μmol photons m^{-2} s^{-1}: 12 h dark) at *in situ* temperature.

We sampled the headspace five times, distributed along the 24 h incubations. Specifically, we withdrew 3 ml of air from the headspace with a gas-tight syringe. The headspace air sample was immediately injected into a 3 ml vacuum vial for further analysis of ethylene concentration on a gas chromatographer equipped with a flame ionization detector and coupled to a mass spectrometer (MS-FID-GC, Agilent 7890) using a GS-CarbonPLOT column (60 m × 320 μm × 1.5 μm, Agilent Technologies, USA). We built a calibration curve using three ethylene standards of known concentration (1.5, 9, and 93 ppm) and Helium as a balance gas, supplied by Abdullah Hashim Industrial Gases & Equipment Co. Ltd. (Jeddah, Saudi Arabia). We estimated the concentration of dissolved ethylene before equilibrium with the headspace, from the ethylene

226 concentration in the equilibrated air according to Wilson et al. (2012) and applying the solubility
227 coefficient of ethylene extracted from Breitbarth et al. (2004) as a function of temperature and
228 salinity.
229 We run the following negative controls at each sampling event: i) roots, shoots, and sediment
230 without addition of acetylene-saturated seawater in order to confirm that ethylene was not
231 naturally produced by our samples, and ii) seawater collected from the study site and used in the
232 preparation of the incubations with addition of acetylene-saturated seawater in order to measure
233 the N_2 fixation due to pelagic diazotrophs. The ethylene production rate measured in the
234 seawater control was subtracted from the ethylene production rates detected in our samples. The
235 net ethylene rates (after subtracting the background seawater rate) were converted into N_2
236 fixation rates by applying the common ratio of 3 mol of acetylene:1 mol of N_2 (Welsh, 2000).
237 At the end of the incubation, we dried the roots, shoots, and sediment samples at 60^{o}C and
238 recorded the dry weight for further calculations. Moreover, we calculated the sediment organic
239 matter (OM) content of each replicate sediment horizon by loss on ignition (Dean Jr, 1974).
240 Then, the sediment N_2 fixation rates were standardized to surface area integrated over 3 cm
241 sediment depth by averaging the rates measured at the first 3 sediment horizons and taking into
242 account the sediment bulk density in order to compare N_2 fixation to denitrification and
243 anammox rates. The N_2 fixation rates of roots and shoot epiphytes were standardized to surface
244 area taking into account the biomass density.
245
246 **2.5. Statistical analysis**
247 Differences in OM content (our continuous response variable) were tested considering the
248 categorical explanatory variables ‘sediment type’ (2 levels: vegetated and bare sediments) with

249 the non-parametric Wilcoxon test. Furthermore, we analyzed OM content considering also as
250 explanatory variable 'sediment horizons' (4 levels: from sediment surface to 1 cm deep, from 1
251 to 2 cm deep, from 2 to 3 cm deep, and from 9 to 10 cm deep), and 'sampling events' (5 levels:
252 June, August, November, February and April) by performing a Generalized Linear Model
253 (GLM) and considering their interaction. All the factors were fixed and orthogonal.
254 Differences in O_2 concentration and H_2S concentration between vegetated and bare sediments
255 and between light and dark were tested by nonparametric Wilcoxon test.
256 Differences in denitrification, anammox, and N_2 fixation rates per gram of sediment along the
257 sediment horizons between vegetated and bare sediments were tested by Wilcoxon matched-
258 pairs signed rank test. Similarly, we test for differences between denitrification, anammox, and
259 N_2 fixation rates by Wilcoxon matched-pairs signed rank test. Moreover, we analyzed the
260 difference in denitrification, anammox, and N_2 fixation rates per gram of sediment considering
261 the type of sediment and the sediment OM content as our categorical and continuous explanatory
262 variables, respectively. Since the distribution of denitrification, anammox, and N_2 fixation rates
263 per gram of sediment was not normal, we used a GLM to test for differences.
264 Finally, we analyzed the difference in depth-integrated denitrification, anammox, and N_2 fixation
265 rates, our continuous response variables, considering the type of sediment and sampling event as
266 our categorical explanatory variables. We furthermore analyzed the difference in depth-
267 integrated denitrification, anammox, and N_2 fixation rates, considering the type of sediment and
268 temperature as our categorical and continuous explanatory variables, respectively. Since the
269 distribution of the depth-integrated denitrification and N_2 fixation rates was not normal, we used
270 a GLM to test for differences, while we used a linear model test to analyze the depth-integrated
271 anammox rates as its distribution was normal. The effect of temperature and sediment type on

272 the net N_2 flux was tested by using a linear model. All statistical analyses were performed using
273 JMP (SAS Institute Inc., USA) and PRISM (GraphPad Software Inc., USA) statistical software.
274
275 **3. Results**
276 **3.1. Water and sediment properties**
277 The *in situ* daily average seawater temperature ranged from 22.3°C in February to 32.5°C in June
278 (Table 1), while annual mean salinity was 41.2 ± 0.4 PSU. The OM content was consistently
279 higher (about 40% higher) in the vegetated sediments compared to the bare sediment
280 (nonparametric Wilcoxon test, $p < 0.0001$), with annual mean (± SEM) OM content of 13.5 ± 0.1
281 and 8.5 ± 0.1% of sediment dry weight, respectively, and decreased with increasing depth (Fig.
282 1a). The sediment OM content significantly differed among sediment type, sampling event, and
283 sediment horizon (GLM; sediment type*sampling event*sediment horizon $\chi^2_{12,80} = 28.7$; $p =$
284 0.004). The maximum depth-integrated mean OM content in vegetated sediments (15% of
285 sediment dry weight) and bare sediments (9.5% of sediment dry weight) was found in November
286 and June, respectively.
287 Sediment O_2 microprofiles significantly differed between vegetated and bare sediments during
288 light and dark measurements (nonparametric Wilcoxon test, $p = 0.0002$ and $p < 0.0001$,
289 respectively) and between light and dark conditions in both vegetated and bare sediments
290 (nonparametric Wilcoxon test, $p < 0.0001$ and $p < 0.0001$, respectively). The vegetated and bare
291 sediments were anoxic below the sediment surface but the sediment depth at which anoxic
292 conditions prevailed varied depending on sediment type, light or dark conditions, and the time of
293 the year (Fig. 1b, c). In vegetated sediments, the anoxic conditions appeared at deeper layers than
294 in the bare sediments. Similarly, O_2 diffused into deeper sediment layers during light incubations

than during dark incubations for both vegetated and bare sediments. On average, the vegetated sediments were anoxic at 1 ± 0.3 cm and 0.7 ± 0.1 cm below the surface under light and dark conditions, respectively, while bare sediments were anoxic at 0.6 ± 0.2 cm and 0.4 ± 0.1 cm below the surface under light and dark conditions, respectively. In some vegetated sediments under light conditions (Fig. 1b1, b3), the O_2 concentration increased again at deep layers, likely indicating O_2 release by seagrass roots into the sediment or O_2 diffusion through animal burrows. Sediment H_2S microprofiles were highly variable along the year (Fig. 1b, c). Under light conditions, the H_2S concentration in bare sediments (median = 1.28 μmol H_2S L^{-1}) was significantly higher than in vegetated sediments (median = 0 μmol H_2S L^{-1}) (nonparametric Wilcoxon test, $p < 0.0001$). Similarly, under dark conditions, the H_2S concentration in bare sediments (median = 1.17 μmol H_2S L^{-1}) was significantly higher than in vegetated sediments (median = 0.008 μmol H_2S L^{-1}) (nonparametric Wilcoxon test, $p < 0.0001$). In vegetated sediments, the H_2S concentration was very low (< 0.5 μM) during the summer months (June and August, Fig. 1b1-2) and the maximum H_2S concentration (10.4 μM) was detected in November under dark conditions (Fig. 1b3) at 2.2 cm below the sediment surface. Bare sediments showed similar H_2S profiles under light and dark conditions, except for the dark measurement in November. The maximum H_2S concentration in bare sediments (15.2 μM) was also detected in November under light conditions, but it was higher than that in vegetated ones and at deeper sediment layers (Fig. 1c3), about 6 cm below the surface. The redox potential ranged from about 550 mV to -450 mV (Fig. S1) and decreased abruptly with increasing sediment depth.

3.2. Denitrification, anammox and N_2 fixation rates

Sediment denitrification rates per gram of sediment were consistently higher in vegetated sediments compared to bare sediments (Wilcoxon matched-pairs signed rank test, $p = 0.0015$, Fig. 2a). The highest denitrification rates were detected in summer (June and August, Fig. 2a1-2) for both vegetated and bare sediments. In vegetated sediments, the maximum denitrification rate (20.52 ± 0.6 nmol N g DW^{-1} h^{-1}) was found in June and was almost twice the maximum rate measured in bare sediments (11.5 ± 4.2 nmol N g DW^{-1} h^{-1}), which was found in August. Although the rates varied throughout the year, the maximum denitrification rates took place between 1 and 2 cm below surface, with minimum rates detected in the sediment surface. Anammox rates per gram of sediment were consistently lower than denitrification rates (Wilcoxon matched-pairs signed rank test, $p < 0.0001$) and without significant difference between vegetated and bare sediments (Wilcoxon matched-pairs signed rank test, $p = 0.6788$, Fig. 2b). In vegetated sediments, the maximum anammox rate (6.88 ± 0.5 nmol N g DW^{-1} h^{-1}) was detected in August and was similar to the maximum rate in bare sediments (6.89 ± 0.4 nmol N g DW^{-1} h^{-1}), measured in April. The minimum denitrification and anammox rates were measured in November. Sediment N_2 fixation rates per gram of sediment (Fig. 2c) were significantly lower than denitrification and anammox rates (Wilcoxon matched-pairs signed rank test, $p < 0.0001$ and $p < 0.0001$, respectively), with maximum N_2 fixation rates (1.25 ± 0.1 nmol N g DW^{-1} h^{-1}) detected in November, in contrast to the denitrification and anammox patterns. The N_2 fixation rates were significantly higher in vegetated sediments than those rates measured in bare sediments (Wilcoxon matched-pairs signed rank test, $p < 0.0001$, Fig. 2c). Denitrification rates per gram of sediment differed between vegetated and bare sediments at different sediment horizons, however sediment OM content did not have a significant effect (GLM; sediment type $\chi^2_{1,28} = 5.6$, $p < 0.05$; OM content $\chi^2_{1,28} = 3.1$, $p = 0.08$) (Fig. 3a). The

sediment OM content and the type of sediment had a significant effect on anammox rates (GLM; sediment type $\chi^2_{1,28}$ = 4.5, $p < 0.05$; OM content $\chi^2_{1,28}$ = 5.1, $p < 0.05$) and N_2 fixation rates (GLM; sediment type*OM content $\chi^2_{1,36}$ = 14.2, $p < 0.001$). Anammox rates decreased with increasing OM content in vegetated sediments ($Y = -1.04X + 17.8$, $p < 0.05$, Fig. 3b), while N_2 fixation rates increased with increasing OM content in vegetated sediment ($Y = 0.24X - 2.9$, $p < 0.0001$, Fig. 3c).

The differences in denitrification rates between vegetated and bare sediment rates became smaller when depth-integrated (0 – 3 cm) rates were compared (Fig. 4a), largely due to the higher (1.5-fold) bulk density in bare sediments compared to vegetated sediments. Depth-integrated denitrification rates significantly differed among sampling events but not between vegetated and bare sediments (GLM; sampling event $\chi^2_{4,24}$ = 70.6, $p < 0.0001$; sediment type $\chi^2_{1,24}$ = 3.1, $p = 0.08$), with minimum rates overserved in November in both sediment types. Depth-integrated anammox rates (Fig. 4b) significantly differed among sampling events and between vegetated and bare sediments (lm, sampling event*sediment type; $F_{4,29}$ = 30.05, $p < 0.0001$). Minimum depth-integrated anammox rates were detected in November in both sediment types, however rates were consistently higher in bare sediments compared to vegetated sediments throughout the year. Similarly, depth-integrated N_2 fixation rates (Fig. 4c) significantly differed among sampling events and between vegetated and bare sediments (GLM, sampling event*sediment type $\chi^2_{4,20}$ = 73.31, $p < 0.0001$), with consistently higher rates in vegetated sediments. Maximum depth-integrated N_2 fixation rates were observed in November in both types of sediments.

3.3. Effect of temperature on denitrification, anammox and N_2 fixation rates

363 Temperature had a significant effect on depth-integrated denitrification rates regardless of the
364 type of sediment (GLM; temperature $\chi^2_{1,27}$ = 16.67, p < 0.0001; sediment type $\chi^2_{1,27}$ = 0.53, p =
365 0.46;). Depth-integrated denitrification rates increased linearly with temperature ($Y = 3.569X -$
366 65, Fig. 5a). Temperature and sediment type had a significant effect on depth-integrated
367 anammox rates (lm; temperature $F_{1,29}$ = 14.8, p = 0.0007; sediment type, $F_{1,29}$ = 7.7, p = 0.01),
368 with rates increasing linearly in vegetated ($Y = 1.3X - 20.36$) and bare ($Y = 1.3X - 16.94$)
369 sediments (Fig. 5b). However, depth-integrated N_2 fixation rates did not increase linearly with
370 temperature and the differences in rates were explained by sediment type (GLM; sediment type
371 $\chi^2_{1,27}$ = 4.93, p = 0.03). Sediment N_2 fixation rates in vegetated and bare sediments showed a
372 different thermal response than denitrification and anammox processes, with maximum rates
373 reported at 28.5ºC and decreasing rates at either lower and higher temperatures (Fig. 5c). N_2
374 fixation rates followed a second-degree polynomial curve ($Y = 16.94 - 0.45X - 0.13X^2$, r^2 =
375 0.40, p < 0.05) in vegetated sediments. N_2 fixation rates in seagrass roots and epiphytes showed
376 the same annual pattern that the rates reported for the rhizosphere. The maximum rates in
377 seagrass roots (21.9 ± 210.7 µg N g DW^{-1} d^{-1}) and epiphytes (10.4 ± 1.5 µg N g DW^{-1} d^{-1}) were
378 also recorded in November when *in situ* seawater temperature was 28.5ºC (Fig. 5c).
379
380 **3.4. Net N_2 fluxes**
381 The net N_2 fluxes ranged from 3.6 ± 0.8 and 19.73 ± 0.9 mg N m^{-2} d^{-1} in November, to 85.1 ±
382 3.7 and 85.1 ± 2.6 mg N m^{-2} d^{-1} in summer months for the seagrass meadow and bare sediments,
383 respectively (Fig. 6). The net N_2 flux significantly differed among sampling events but not
384 between sediment type (lm; sampling event $F_{4,9}$ = 24.76, p = 0.004; sediment type, $F_{1,9}$ = 1.83, p

385 = 0.25). Net N_2 flux increased linearly with temperature ($Y = 4.99X - 91.86$, $r^2 = 0.43$, $p <$

386 0.05, Fig. 7).

387

388 **4. Discussion**

389 The sediment organic matter content in the Red Sea lagoon system studied here was extremely

390 high, especially in the vegetated sediments (mean = 13.5%), compared to the values reported for

391 other seagrass sediments (mean = 4.1%, Kennedy et al., 2010). The higher sediment organic

392 matter content in vegetated sediments, compared to bare sediments, corroborates the evidence

393 that seagrasses accumulate and store large amounts of organic carbon in their sediments (Duarte

394 et al., 2005). In addition, seagrasses modified the physicochemical conditions of the sediment

395 (Enriquez et al., 2001), as reflected in higher O_2 and lower sulfide sediment concentrations than

396 those in the adjacent bare sediment. Moreover, O_2 profiles showed higher variability in vegetated

397 sediments. This can be directly related to bioturbation (Kristensen et al., 2012) and to the radial

398 oxygen loss by roots leading to oxic layers at depth (Pedersen et al., 1998), enhancing the

399 complexity and heterogeneity of seagrass sediments.

400 The denitrification rate in *E. acoroides* sediments reported here (annual mean = 34.9 ± 10.3 mg

401 N m^{-2} d^{-1}) is 6-fold higher than the rate reported for a restored *Zostera marina* meadow in

402 Virginia using an *in situ* push-pull incubation method (Aoki and McGlathery, 2017), 1.3 to 2.5-

403 fold higher than the rate previously reported for tropical meadows dominated by *E. acoroides* on

404 slurries from the top 5 cm sediment (Alongi et al., 2008), comparable to the rates reported for

405 temperate seagrasses (Eyre et al., 2016), and 8-fold lower than the rates reported for sub-tropical

406 estuarine seagrasses (Eyre et al., 2011a) using *in situ* benthic chambers. However, the use of the

407 ^{15}N isotope pairing technique on sediment slurries could have underestimated denitrification

408 rates reported here. Measuring N_2 fluxes on intact sediment cores has been proved to better
409 account for coupled nitrification and denitrification than the ^{15}N isotope pairing technique (van
410 Luijn et al., 1996). In addition, sediment slurries disturb the sediment structure and redox
411 gradient, and, therefore, might prevent the coupled nitrification and denitrification in the
412 transition layers from oxic to anoxic conditions (Eyre et al., 2002;Herbert, 1999). Since the
413 coupled nitrification and denitrification has been reported to be important in continental shelf
414 and coastal sediments (Herbert, 1999;Gardner and McCarthy, 2009;Christensen et al., 1987); the
415 denitrification rates in this coastal lagoon could be higher than actual reported values.
416 Overall, the observed denitrification rates were higher in vegetated sediments than bare
417 sediments when expressed per gram of dried sediment. However, we did not find differences
418 between depth-integrated denitrification rates in vegetated and bare sediments (annual mean =
419 34.9 ± 10.3 and 31.6 ± 8.9 mg N m^{-2} d^{-1}, respectively) contrary to previous findings (Eyre et al.,
420 2011b).
421 The potential sediment anammox rates reported here, ranging from 0.5 to 6.9 nmol N g DW^{-1} h^{-1},
422 are comparable to potential anammox rates reported elsewhere (i.e. from 0 to 8 nmol N cm^{-3} h^{-1}
423 in subtropical mangrove sediments (Meyer et al., 2005); from <0.5 to 7.6 nmol N g DW^{-1} h^{-1} in
424 marine sediments from a Fjord in Sweden (Brandsma et al., 2011) and from 0 to 3 nmol N cm^{-3}
425 h^{-1} in sandy sediments in the southern North Sea (Bale et al., 2014)). However, the potential
426 sediment anammox rates detected here (annual mean depth-integrated anammox rates = 12.4 ±
427 3.4 and 19.8 ± 4.4 mg N m^{-2} d^{-1} in vegetated and bare sediments, respectively) are higher than
428 the only estimate available, to the best of our knowledge, for a seagrass ecosystem (0.06 mg N
429 m^{-2} d^{-1} in bare sediment adjacent to a sub-tropical seagrass meadow in Australia on intact cores
430 (Salk et al., 2017)). The contribution of anammox to the total loss of N reported here (27 and

38% in vegetated and bare sediments, respectively, Fig. S2) is smaller than the contribution reported by Salk et al. (Salk et al., 2017), with anammox accounting for 64 to 86% of the total N loss, but still within the range of anammox-supported N losses reported for other marine sediments (Devol, 2015;Bale et al., 2014).

The maximum N_2 fixation rates reported for *E. acoroides* sediments here (6.3 ± 0.5 mg N m^{-2} d^{-1}) are lower than the previously reported maximum N_2 fixation rates in sediments of a tropical mixed meadow dominated by *E. acoroides* (19.4 ± 3.2 mg N m^{-2} d^{-1}, (Alongi et al., 2008)). Similarly, Moriarty and O'Donohue (1993) reported higher N_2 fixation rates for a mixed meadow dominated by *E. acoroides* (25 ± 2.6 mg N m^{-2} d^{-1}) than those reported here during the same time of the year (16.4 ± 0.4 mg N m^{-2} d^{-1},) although with a smaller contribution from leaf epiphytes (4.2 ± 0.1 mg N m^{-2} d^{-1}) compared with our N_2 fixation rates from epiphytes (7.9 ± 1.1 mg N m^{-2} d^{-1}). The N_2 fixation rates supported by roots are in agreement with previous findings of N_2-fixing bacteria in association with seagrass roots (Garcias-Bonet et al., 2012;Garcias-Bonet et al., 2016). Moreover, the N_2 fixation rates previously reported for surface-sterilized *E. acoroides* roots (0.13 mg N m^{-2} d^{-1} (Raja et al., 2012)) are 17-fold lower than the rates reported here (2.3 ±1.5 mg N m^{-2} d^{-1}) for the same seawater temperature (29ºC), pointing out at the role of bacteria inhabiting the rhizoplane of *E. acoroides* roots in nutrient supply. Despite the common use of the ARA to measure N_2 fixation in natural communities, such as open ocean waters (i.e. Falcón et al., 2004) and vegetated coastal sediments (i.e. Eyre et al., 2011a), including seagrass sediments (see references in Welsh, 2000), and seagrass tissues (Nielsen et al., 2001;Raja et al., 2012), it has some methodological limitations that need to be considered. Acetylene is known to induce changes in the biogeochemistry and the microbial community composition in marine sediments, especially affecting sulfur- and sulfate-reducing bacterial

groups (Fulweiler et al., 2015). However, the effect of acetylene is species specific, and, therefore, the N_2 fixation rates reported here might be either under- or over- estimated and need to be carefully interpreted.

The highest N_2 fixation rates in vegetated and bare sediments coincided with the highest sediment sulfide concentrations (10.4 and 15.2 μmol H_2S L^{-1} in vegetated and bare sediments, respectively), suggesting a contribution of sulfate-reducing bacteria to the pool of N_2-fixing bacteria, as reported in other seagrass rhizospheres (Welsh et al., 1996). Although sulfide has detrimental effects on seagrasses (Garcias-Bonet et al., 2008), the sulfide concentrations in the vegetated sediments were generally below the threshold limit of 10 μmol H_2S L^{-1} for seagrass decline (Calleja et al., 2007).

The contrasting annual patterns in denitrification and anammox compared to those of N_2 fixation, with highest rates of denitrification and anammox in summer and spring while maximum N_2 fixation in autumn, suggest differential specific thermal responses. The linear increase of denitrification and anammox with temperature found here was already described for net sediment N_2 fluxes in estuaries (Nowicki, 1994). Similarly, the thermal response of N_2 fixation found here, with maximum rates at intermediate temperatures (29ºC) and a decrease in rates at lower and higher temperatures, is in agreement with the notable decrease in N_2 fixation rates at 33 and 35ºC reported for Mediterranean macrophytes (Garcias-Bonet et al., 2018) and cyanobacteria in soil crusts (Zhou et al. 2016), respectively. Moreover, these different annual patterns could be partially explained by changes in sediment OM. The sediment microbial activity is modulated, as well, by the quantity and quality of the OM. For instance, decomposition and remineralization rates of OM depends on its lability (Herbert, 1999) which is indicated by the C:N:P ratio that differs among sources (Enríquez et al., 1993). OM from phytoplankton decomposes faster than

477 OM from seagrasses, due to their higher N content and therefore lower C:N:P ratios. Eyre et al.
478 (2013) demonstrated that the source of the OM, and therefore, its C:N ratio controls
479 denitrification rates in coastal sediments. Tibbles et al. (1994) showed an increase in sediment N_2
480 fixation following the addition of complex plant polysaccharides and Fulweiler et al. (2013)
481 argued that an increase in the C:N ratio of OM was responsible for the decrease in denitrification
482 and the increase in N_2 fixation, in agreement with the effect of OM reported here.
483 The net N_2 fluxes in the Red Sea lagoon ecosystem indicates this ecosystem supports a net loss
484 of reactive N as N_2 gas throughout the year, as sediment denitrification and anammox
485 consistently exceeded N_2 fixation in sediment and seagrass tissues. Alongi et al. (2008) also
486 reported higher denitrification than N_2 fixation rates in an *E. acoroides* meadow. Integrating the
487 average seasonal rates, we estimate the annual N loss in 14.9 g N m^{-2} yr^{-1} in the seagrass
488 meadow and 18.2 g N m^{-2} yr^{-1} in bare sediments. Despite the lack of rivers discharging into the
489 Red Sea, the occasional heavy rains, groundwater discharge, and atmospheric deposition might
490 lead to high reactive N loads reaching coastal systems (Voss et al., 2013). Therefore, the high
491 denitrification and anammox rates in this coastal lagoon provide a mechanism adding resistance
492 to excess N inputs (Zarnoch et al., 2017;Galloway et al., 2003), which might lead to permanent
493 seagrass losses due to hypoxia after algal blooms produced by severe eutrophication (Herbert,
494 1999;Duarte, 1995). However, dissimilatory nitrate reduction to ammonium (DNRA) competes
495 with denitrification by reducing nitrate availability. In a shallow estuary, DNRA was identified
496 as an important nitrate loss pathway, with rates comparable to denitrification rates (An and
497 Gardner, 2002); and in a restored *Zostera marina* meadow, DNRA accounted for 45 % of
498 sediment nitrate reduction (Aoki and McGlathery, 2017). Therefore, the net N loss reported here
499 could be lower due to a potential limitation of denitrification.

Nevertheless, N_2 fixation plays an important role in supporting seagrass meadows in the Red Sea, as the maximum N_2 fixation rate reported here could contribute from 7 to 36.4% of the N requirements to support *E. acoroides* growth, calculated using previous estimates of biomass productivity (Alongi et al., 2008;Pedersen et al., 2016) and measured N content of leaf tissues in this central Red Sea lagoon (Almahasheer et al., 2017). Furthermore, the N isotopic composition reported for *E. acoroides* tissues in this location ($\delta^{15}N_{leaves}$ = 0.17‰ and $\delta^{15}N_{rhizomes}$ = -1.56‰ (Almahasheer et al., 2017)), provides evidence for the atmospheric origin of the assimilated N. The differential apparent thermal response of denitrification and anammox, which increased with increasing temperature, and N_2 fixation, which showed a maximum at about 28ºC, leads to an increase in the net N_2 flux with temperature (fig. 6). This has important implications in a context of rapid warming of the Red Sea (Chaidez et al. 2017). In particular, a further increase in temperature, with maximum temperatures in excess of 33ºC at present (Chaidez et al. 2017), might lead to a further imbalance in N cycling in tropical seagrass ecosystems, similar to that predicted for soil crusts (Zhou et al., 2016). Therefore, the forecasted warming might have an important impact on N availability and therefore on seagrass productivity and their capacity to mitigate climate change.

Conclusion

The studied coastal lagoon ecosystem supported a net loss of reactive N as N_2, with anammox accounting for about one-third of N_2 production. However, N_2 fixation supported part of seagrass growth. The results presented suggest that, as a consequence of the differential thermal responses of processes supporting losses and gains of reactive N, future warming can enhance the role of seagrass meadows as sites of reactive N loss in an already warm Red Sea.

Author contribution

NG-B and CMD designed the study. NG-B and MF performed the fieldwork. NG-B performed the N_2 fixation measurements. MF performed the sediment microprofiles. MA and DRS performed the denitrification and anammox activity measurements. NG-B, MF and CMD interpreted the results. NG-B wrote the first draft of the manuscript. All authors contributed substantially to the final manuscript.

Competing interests

The authors declare that they have no conflict of interest.

Acknowledgements

This research was funded by King Abdullah University of Science and Technology through Baseline funding to C.M.D, D.D. and P.E. S. We thank Mongi Ennasri for his support in sample analysis.

References

Almahasheer, H., Serrano, O., Duarte, C. M., Arias-Ortiz, A., Masque, P., and Irigoien, X.: Low Carbon sink capacity of Red Sea mangroves, Scientific Reports, 7, 9700, 2017.

Alongi, D. M., Trott, L. A., Undu, M. C., and Tirendi, F.: Benthic microbial metabolism in seagrass meadows along a carbonate gradient in Sulawesi, Indonesia, Aquatic Microbial Ecology, 51, 141-152, 10.3354/ame01191, 2008.

Amano, T., Yoshinaga, I., Okada, K., Yamagishi, T., Ueda, S., Obuchi, A., Sako, Y., and Suwa, Y.: Detection of anammox activity and diversity of anammox bacteria-related 16S rRNA genes in coastal marine sediment in Japan, Microbes and Environments, 22, 232-242, 2007.

Amano, T., Yoshinaga, I., Yamagishi, T., Van Thuoc, C., Ueda, S., Kato, K., Sako, Y., and Suwa, Y.: Contribution of anammox bacteria to benthic nitrogen cycling in a mangrove forest and shrimp ponds, Haiphong, Vietnam, Microbes and environments, 26, 1-6, 2011.

An, S., and Gardner, W. S.: Dissimilatory nitrate reduction to ammonium (DNRA) as a nitrogen link, versus denitrification as a sink in a shallow estuary (Laguna Madre/Baffin Bay, Texas), Marine Ecology Progress Series, 237, 41-50, 2002.

Aoki, L. R., and McGlathery, K. J.: Push-pull incubation method reveals the importance of denitrification and dissimilatory nitrate reduction to ammonium in seagrass root zone, Limnology and Oceanography: Methods, 15, 766-781, 2017.

Bale, N. J., Villanueva, L., Fan, H., Stal, L. J., Hopmans, E. C., Schouten, S., and Sinninghe Damsté, J. S.: Occurrence and activity of anammox bacteria in surface sediments of the southern North Sea, FEMS microbiology ecology, 89, 99-110, 2014.

Brandsma, J., van de Vossenberg, J., Risgaard-Petersen, N., Schmid, M. C., Engström, P., Eurenius, K., Hulth, S., Jaeschke, A., Abbas, B., and Hopmans, E. C.: A multi-proxy study of anaerobic ammonium oxidation in marine sediments of the Gullmar Fjord, Sweden, Environmental microbiology reports, 3, 360-366, 2011.

Breitbarth, E., Mills, M. M., Friedrichs, G., and LaRoche, J.: 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, 282-288, 2004.

Calleja, M., Marba, N., and Duarte, C. M.: The relationship between seagrass (Posidonia oceanica) decline and porewater sulfide pools in carbonate sediments., Estuarine, Coastal and Shelf Science, 73, 583-588, 2007.

Capone, D. G., and Taylor, B. F.: N2 FIXATION IN THE RHIZOSPHERE OF THALASSIA-TESTUDINUM, Can. J. Microbiol., 26, 998-1005, 1980.

Chaidez, V., Dreano, D., Agusti, S., Duarte, C. M., and Hoteit, I.: Decadal trends in Red Sea maximum surface temperature, Scientific Reports, 7, 8144, 2017.

Christensen, J. P., Murray, J. W., Devol, A. H., and Codispoti, L. A.: Denitrification in continental shelf sediments has major impact on the oceanic nitrogen budget, Glob. Biogeochem. Cycle, 1, 97-116, 1987.

Dalsgaard, T., Thamdrup, B., and Canfield, D. E.: Anaerobic ammonium oxidation (anammox) in the marine environment, Res. Microbiol., 156, 457-464, http://dx.doi.org/10.1016/j.resmic.2005.01.011, 2005.

Dean Jr, W. E.: 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, 1974.

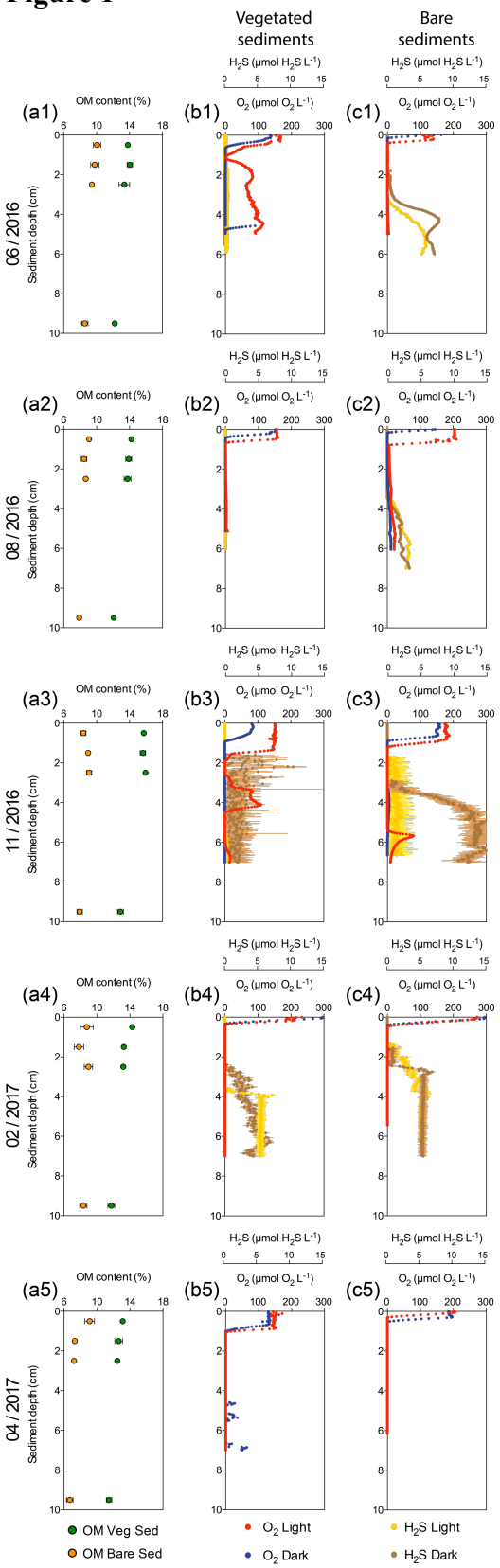
582 Devol, A. H.: Denitrification, anammox, and N2 production in marine sediments, Annual review
583 of marine science, 7, 403-423, 2015.
584 Duarte, C. M.: Submerged aquatic vegetation in relation to different nutrient regimes, Ophelia,
585 41, 87-112, 1995.
586 Duarte, C. M., Middelburg, J. J., and Caraco, N.: Major role of marine vegetation on the oceanic
587 carbon cycle, Biogeosciences, 2, 1-8, 2005.
588 Enriquez, S., Marba, N., Duarte, C. M., van Tussenbroek, B. I., and Reyes-Zavala, G.: Effects of
589 seagrass Thalassia testudinum on sediment redox, Marine Ecology-Progress Series, 219,
590 149-158, 2001.
591 Enríquez, S., Duarte, C. M., and Sand-Jensen, K.: Patterns in decomposition rates among
592 photosynthetic organisms: the importance of detritus C: N: P content, Oecologia, 94, 457-
593 471, 1993.
594 Eyre, B. D., Rysgaard, S., Dalsgaard, T., and Christensen, P. B.: Comparison of isotope pairing
595 and N2: Ar methods for measuring sediment denitrification—assumption, modifications,
596 and implications, Estuaries, 25, 1077-1087, 2002.
597 Eyre, B. D., Ferguson, A. J. P., Webb, A., Maher, D., and Oakes, J. M.: Denitrification, N-
598 fixation and nitrogen and phosphorus fluxes in different benthic habitats and their
599 contribution to the nitrogen and phosphorus budgets of a shallow oligotrophic sub-
600 tropical coastal system (southern Moreton Bay, Australia), Biogeochemistry, 102, 111-
601 133, 10.1007/s10533-010-9425-6, 2011a.
602 Eyre, B. D., Maher, D., Oakes, J. M., Erler, D. V., and Glasby, T. M.: Differences in benthic
603 metabolism, nutrient fluxes, and denitrification in Caulerpa taxifolia communities
604 compared to uninvaded bare sediment and seagrass (Zostera capricorni) habitats,
605 Limnology and Oceanography, 56, 1737-1750, 10.4319/lo.2011.56.5.1737, 2011b.
606 Eyre, B. D., Maher, D. T., and Squire, P.: Quantity and quality of organic matter (detritus) drives
607 N-2 effluxes (net denitrification) across seasons, benthic habitats, and estuaries, Glob.
608 Biogeochem. Cycle, 27, 1083-1095, 10.1002/2013gb004631, 2013.
609 Eyre, B. D., Maher, D. T., and Sanders, C.: The contribution of denitrification and burial to the
610 nitrogen budgets of three geomorphically distinct Australian estuaries: Importance of
611 seagrass habitats, Limnology and Oceanography, 2016.
612 Falcón, L. I., Carpenter, E. J., Cipriano, F., Bergman, B., and Capone, D. G.: N2 fixation by
613 unicellular bacterioplankton from the Atlantic and Pacific Oceans: phylogeny and in situ
614 rates, Applied and Environmental Microbiology, 70, 765-770, 2004.
615 Field, C. B., Behrenfeld, M. J., Randerson, J. T., and Falkowski, P.: Primary production of the
616 biosphere: integrating terrestrial and oceanic components, Science, 281, 237-240, 1998.
617 Fowler, D., Coyle, M., Skiba, U., Sutton, M. A., Cape, J. N., Reis, S., Sheppard, L. J., Jenkins,
618 A., Grizzetti, B., and Galloway, J. N.: The global nitrogen cycle in the twenty-first
619 century, Phil. Trans. R. Soc. B, 368, 20130164, 2013.
620 Fulweiler, R., Brown, S., Nixon, S., and Jenkins, B.: Evidence and a conceptual model for the
621 co-occurrence of nitrogen fixation and denitrification in heterotrophic marine sediments,
622 Marine Ecology Progress Series, 482, 57-68, 2013.
623 Fulweiler, R. W., Heiss, E. M., Rogener, M. K., Newell, S. E., LeCleir, G. R., Kortebein, S. M.,
624 and Wilhelm, S. W.: Examining the impact of acetylene on N-fixation and the active
625 sediment microbial community, Frontiers in microbiology, 6, 418, 2015.

626 Galloway, J. N., Aber, J. D., Erisman, J. W., Seitzinger, S. P., Howarth, R. W., Cowling, E. B.,
627 and Cosby, B. J.: The Nitrogen Cascade, BioScience, 53, 341-356, 10.1641/0006-
628 3568(2003)053[0341:TNC]2.0.CO;2, 2003.
629 Garcias-Bonet, N., Marba, N., Holmer, M., and Duarte, C. M.: Effects of sediment sulfides on
630 seagrass Posidonia oceanica meristematic activity, Marine Ecology-Progress Series, 372,
631 1-6, 2008.
632 Garcias-Bonet, N., Arrieta, J. M., de Santana, C. N., Duarte, C. M., and Marbà, N.: Endophytic
633 bacterial community of a Mediterranean marine angiosperm (Posidonia oceanica),
634 Frontiers in Microbiology, 3, 342, 10.3389/fmicb.2012.00342, 2012.
635 Garcias-Bonet, N., Arrieta, J. M., Duarte, C. M., and Marba, N.: Nitrogen-fixing bacteria in
636 Mediterranean seagrass (Posidonia oceanica) roots, Aquatic Botany, 131, 57-60,
637 10.1016/j.aquabot.2016.03.002, 2016.
638 Garcias-Bonet, N., Vaquer-Sunyer, R., Duarte, C. M., and Marba, N.: Warming effect on
639 nitrogen fixation in Mediterranean macrophyte sediments, Biogeosciences Discuss.,
640 2018.
641 Gardner, W. S., and McCarthy, M. J.: Nitrogen dynamics at the sediment–water interface in
642 shallow, sub-tropical Florida Bay: why denitrification efficiency may decrease with
643 increased eutrophication, Biogeochemistry, 95, 185-198, 2009.
644 Hansen, J. W., Udy, J. W., Perry, C. J., Dennison, W. C., and Lomstein, B. A.: Effect of the
645 seagrass Zostera capricorni on sediment microbial processes, Marine Ecology Progress
646 Series, 199, 83-96, 10.3354/meps199083, 2000.
647 Hemminga, M., Harrison, P., and Van Lent, F.: The balance of nutrient losses and gains in
648 seagrass meadows, Marine Ecology Progress Series, 85-96, 1991.
649 Herbert, R. A.: Nitrogen cycling in coastal marine ecosystems, Fems Microbiol. Rev., 23, 563-
650 590, 1999.
651 Holtappels, M., Lavik, G., Jensen, M. M., and Kuypers, M. M.: 15N-labeling experiments to
652 dissect the contributions of heterotrophic denitrification and anammox to nitrogen
653 removal in the OMZ waters of the ocean, in: Methods in enzymology, Elsevier, 223-251,
654 2011.
655 Howarth, R. W.: Nutrient limitation of net primary production in marine ecosystems, Annual
656 review of ecology and systematics, 19, 89-110, 1988.
657 Howarth, R. W., and Marino, R.: Nitrogen as the limiting nutrient for eutrophication in coastal
658 marine ecosystems: evolving views over three decades, Limnology and Oceanography,
659 51, 364-376, 2006.
660 Isobe, K., Koba, K., Ueda, S., Senoo, K., Harayama, S., and Suwa, Y.: A simple and rapid
661 GC/MS method for the simultaneous determination of gaseous metabolites, J. Microbiol.
662 Methods, 84, 46-51, 2011a.
663 Isobe, K., Suwa, Y., Ikutani, J., Kuroiwa, M., Makita, T., Takebayashi, Y., Yoh, M., Otsuka, S.,
664 Senoo, K., and Ohmori, M.: Analytical techniques for quantifying 15N/14N of nitrate,
665 nitrite, total dissolved nitrogen and ammonium in environmental samples using a gas
666 chromatograph equipped with a quadrupole mass spectrometer, Microbes and
667 environments, 26, 46-53, 2011b.
668 Kennedy, H., Beggins, J., Duarte, C. M., Fourqurean, J. W., Holmer, M., Marbà, N., and
669 Middelburg, J. J.: Seagrass sediments as a global carbon sink: Isotopic constraints, Glob.
670 Biogeochem. Cycle, 24, 2010.

671 Kristensen, E., Penha-Lopes, G., Delefosse, M., Valdemarsen, T., Quintana, C. O., and Banta, G.
672 T.: What is bioturbation? The need for a precise definition for fauna in aquatic sciences,
673 Marine Ecology Progress Series, 446, 285-302, 2012.
674 McGlathery, K. J.: Seagrass habitats, in: Nitrogen in the Marine Environment, 2nd ed., edited by:
675 Capone, D. G., Bronk, D. A., Mulholland, M. R., and Carpenter, E. J., Elsevier Academic
676 Press Inc, 525 B Street, Suite 1900, San Diego, Ca 92101-4495 USA, 2008.
677 Meyer, R. L., Risgaard-Petersen, N., and Allen, D. E.: Correlation between anammox activity
678 and microscale distribution of nitrite in a subtropical mangrove sediment, Applied and
679 environmental microbiology, 71, 6142-6149, 2005.
680 Moriarty, D., and O'Donohue, M.: Nitrogen fixation in seagrass communities during summer in
681 the Gulf of Carpentaria, Australia, Marine and Freshwater Research, 44, 117-127,
682 http://dx.doi.org/10.1071/MF9930117, 1993.
683 Nielsen, L. B., Finster, K., Welsh, D. T., Donelly, A., Herbert, R. A., de Wit, R., and Lomstein,
684 B. A.: Sulphate reduction and nitrogen fixation rates associated with roots, rhizomes and
685 sediments from Zostera noltii and Spartina maritima meadows, Environmental
686 Microbiology, 3, 63-71, 2001.
687 Nowicki, B. L.: The effect of temperature, oxygen, salinity, and nutrient enrichment on estuarine
688 denitrification rates measured with a modified nitrogen gas flux technique, Estuarine,
689 Coastal and Shelf Science, 38, 137-156, 1994.
690 Pedersen, O., Borum, J., Duarte, C. M., and Fortes, M. D.: Oxygen dynamics in the rhizosphere
691 of Cymodocea rotundata, Marine Ecology-Progress Series, 169, 283-288, 1998.
692 Pedersen, O., Colmer, T. D., Borum, J., Zavala-Perez, A., and Kendrick, G. A.: Heat stress of
693 two tropical seagrass species during low tides–impact on underwater net photosynthesis,
694 dark respiration and diel in situ internal aeration, New Phytologist, 2016.
695 Raitsos, D. E., Pradhan, Y., Brewin, R. J. W., Stenchikov, G., and Hoteit, I.: Remote Sensing the
696 Phytoplankton Seasonal Succession of the Red Sea, PLOS ONE, 8, e64909,
697 10.1371/journal.pone.0064909, 2013.
698 Raja, S., Thangaradjou, T., Sivakumar, K., and Kannan, L.: Rhizobacterial population density
699 and nitrogen fixation in seagrass community of Gulf of Mannar, India, J.Environ.Biol.,
700 33, 1033-1037, 2012.
701 Salk, K. R., Erler, D. V., Eyre, B. D., Carlson-Perret, N., and Ostrom, N. E.: Unexpectedly high
702 degree of anammox and DNRA in seagrass sediments: Description and application of a
703 revised isotope pairing technique, Geochimica et Cosmochimica Acta, 211, 64-78, 2017.
704 Seitaj, D., Schauer, R., Sulu-Gambari, F., Hidalgo-Martinez, S., Malkin, S. Y., Burdorf, L. D.,
705 Slomp, C. P., and Meysman, F. J.: Cable bacteria generate a firewall against euxinia in
706 seasonally hypoxic basins, Proceedings of the National Academy of Sciences, 112,
707 13278-13283, 2015.
708 Seitzinger, S., Harrison, J., Dumont, E., Beusen, A. H., and Bouwman, A.: Sources and delivery
709 of carbon, nitrogen, and phosphorus to the coastal zone: An overview of Global Nutrient
710 Export from Watersheds (NEWS) models and their application, Glob. Biogeochem.
711 Cycle, 19, 2005.
712 Thamdrup, B., and Dalsgaard, T.: Production of N2 through anaerobic ammonium oxidation
713 coupled to nitrate reduction in marine sediments, Applied and environmental
714 microbiology, 68, 1312-1318, 2002.

715 Tibbles, B., Lucas, M., Coyne, V., and Newton, S.: Nitrogenase activity in marine sediments
716 from a temperate saltmarsh lagoon: modulation by complex polysaccharides, ammonium
717 and oxygen, Journal of experimental marine biology and ecology, 184, 1-20, 1994.
718 van Luijn, F., Boers, P. C., and Lijklema, L.: Comparison of denitrification rates in lake
719 sediments obtained by the N2 flux method, the 15N isotope pairing technique and the
720 mass balance approach, Water Research, 30, 893-900, 1996.
721 Vitousek, P. M., Aber, J. D., Howarth, R. W., Likens, G. E., Matson, P. A., Schindler, D. W.,
722 Schlesinger, W. H., and Tilman, D. G.: Human alteration of the global nitrogen cycle:
723 sources and consequences, Ecol. Appl., 7, 737-750, 1997.
724 Voss, M., Bange, H. W., Dippner, J. W., Middelburg, J. J., Montoya, J. P., and Ward, B.: The
725 marine nitrogen cycle: recent discoveries, uncertainties and the potential relevance of
726 climate change, Philosophical Transactions of the Royal Society of London B: Biological
727 Sciences, 368, 20130121, 2013.
728 Weikert, H.: Plankton and the pelagic environment, in: Red Sea, edited by: Edwards, A. J., and
729 Head, S. M., Pergamon Press, Oxford 90-111, 1987.
730 Welsh, D. T., Bourgues, S., deWit, R., and Herbert, R. A.: Seasonal variations in nitrogen-
731 fixation (acetylene reduction) and sulphate-reduction rates in the rhizosphere of Zostera
732 noltii: Nitrogen fixation by sulphate reducing bacteria, Marine Biology, 125, 619-628,
733 1996.
734 Welsh, D. T.: Nitrogen fixation in seagrass meadows: Regulation, plant-bacteria interactions and
735 significance to primary productivity, Ecology Letters, 3, 58-71, 2000.
736 Welsh, D. T., Bartoli, M., Nizzoli, D., Castaldelli, G., Riou, S. A., and Viaroli, P.:
737 Denitrification, nitrogen fixation, community primary productivity and inorganic-N and
738 oxygen fluxes in an intertidal Zostera noltii meadow, Marine Ecology-Progress Series,
739 208, 65-77, 2000.
740 Welsh, D. T.: It's a dirty job but someone has to do it: the role of marine benthic macrofauna in
741 organic matter turnover and nutrient recycling to the water column, Chemistry and
742 Ecology, 19, 321-342, 2003.
743 Wilson, S. T., Böttjer, D., Church, M. J., and Karl, D. M.: Comparative Assessment of Nitrogen
744 Fixation Methodologies, Conducted in the Oligotrophic North Pacific Ocean, Applied
745 and Environmental Microbiology, 78, 6516-6523, 10.1128/aem.01146-12, 2012.
746 Yoshinaga, I., Amano, T., Yamagishi, T., Okada, K., Ueda, S., Sako, Y., and Suwa, Y.:
747 Distribution and diversity of anaerobic ammonium oxidation (anammox) bacteria in the
748 sediment of a eutrophic freshwater lake, Lake Kitaura, Japan, Microbes and
749 environments, 26, 189-197, 2011.
750 Zarnoch, C. B., Hoellein, T. J., Furman, B. T., and Peterson, B. J.: Eelgrass meadows, Zostera
751 marina (L.), facilitate the ecosystem service of nitrogen removal during simulated
752 nutrient pulses in Shinnecock Bay, New York, USA, Marine pollution bulletin, 124, 376-
753 387, 2017.
754 Zhou, X., Smith, H., Silva, A. G., Belnap, J., and Garcia-Pichel, F.: Differential responses of
755 dinitrogen fixation, diazotrophic cyanobacteria and ammonia oxidation reveal a potential
756 warming-induced imbalance of the N-cycle in biological soil crusts, PloS one, 11,
757 e0164932, 2016.
758

759 **Figure 1**



760

Fig. 1. Characterization of *Enhalus acoroides* seagrass vegetated sediments and adjacent bare sediments at five samplings times along the year. **a1-5**. Sediment organic matter content in vegetated (green dots) and bare (orange dots) sediment horizons. **b1-5**. Vegetated sediment O_2 microprofiles under light (red) and dark (blue) incubations and H_2S microprofiles during light (yellow) and dark (brown) incubations (no data available for H_2S profiles on the last sampling). **c1-5**. Bare sediment O_2 microprofiles under light (light red) and dark (dark red) incubations and H_2S microprofiles under light (yellow) and dark (brown) incubations (no data available for H_2S profiles on the last sampling).

Fig. 2

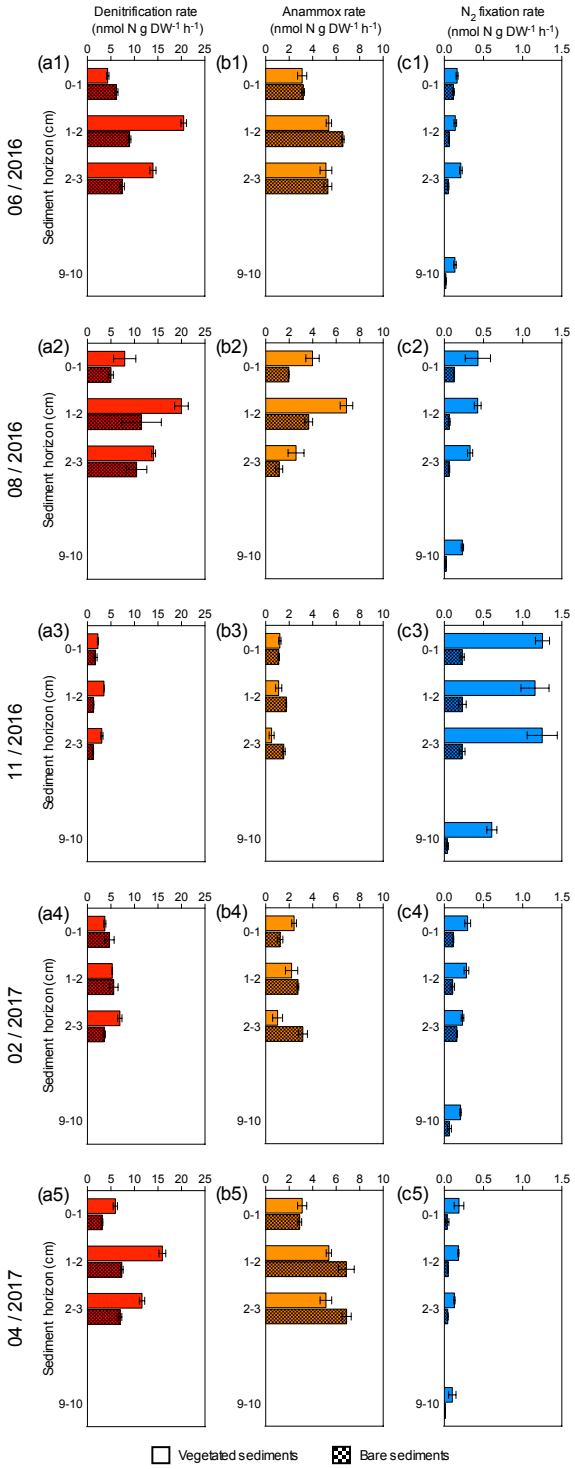
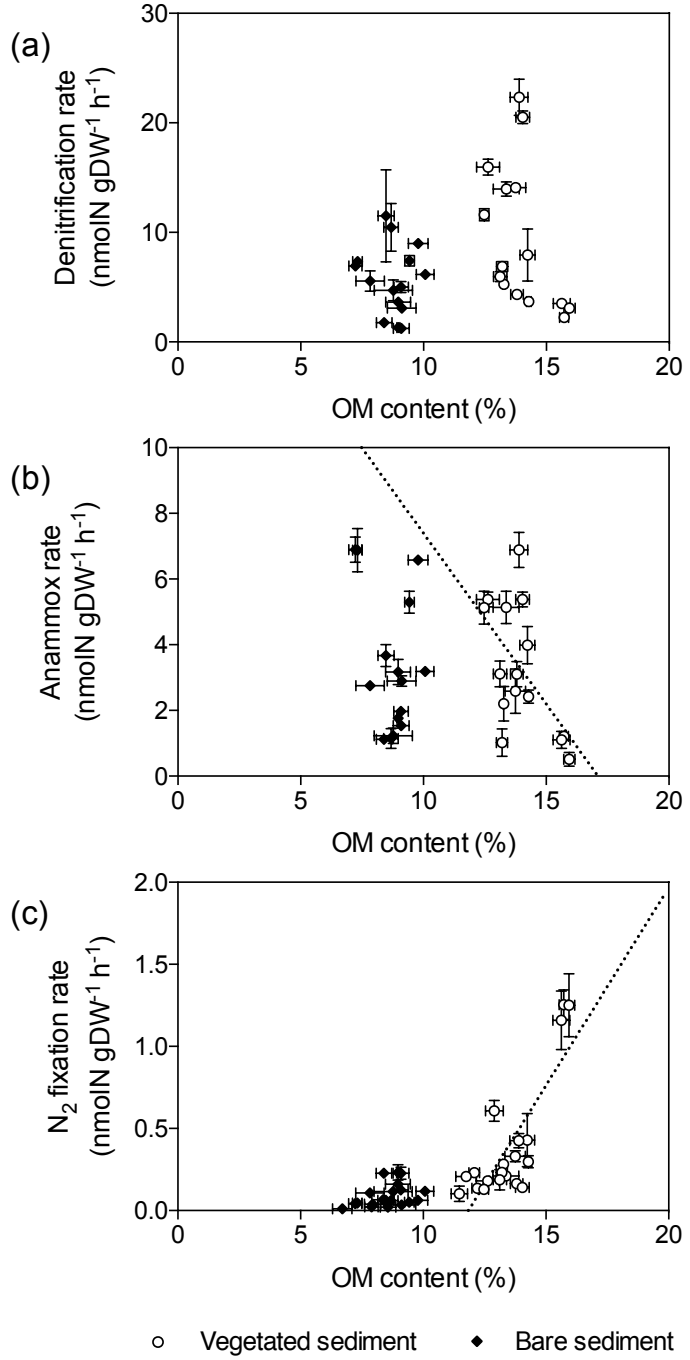


Fig. 2. Sediment profiles of denitrification, anammox and N_2 fixation rates at five samplings times. **a1-5**. Sediment denitrification rates in vegetated (red) and bare (red square pattern) sediment horizons. **b1-5**. Sediment anammox rates in vegetated (orange) and bare (orange square pattern) sediment horizons. **c1-5**. Sediment N_2 fixation rates in vegetated (blue) and bare (blue square pattern) sediment horizons. Error bars indicate SEM.

778 **Fig. 3**



779
780 **Fig. 3.** Relation of denitrification, anammox, and N_2 fixation rates with sediment OM content. **a**.
781 Denitrification rates in vegetated sediments (white dots) and bare sediments (black diamonds). **b**.
782 Anammox rates in vegetated (white dots) and bare (black diamonds) sediments, showing the
783 linear decrease of anammox rates in vegetated sediments with increasing OM content (dotted
784 line) **c**. N_2 fixation rates in vegetated sediments (white dots) and bare sediments (black
785 diamonds), showing the linear increase of N_2 fixation rates in vegetated sediments with
786 increasing OM content (dotted line). Error bars indicate SEM.

Fig. 4

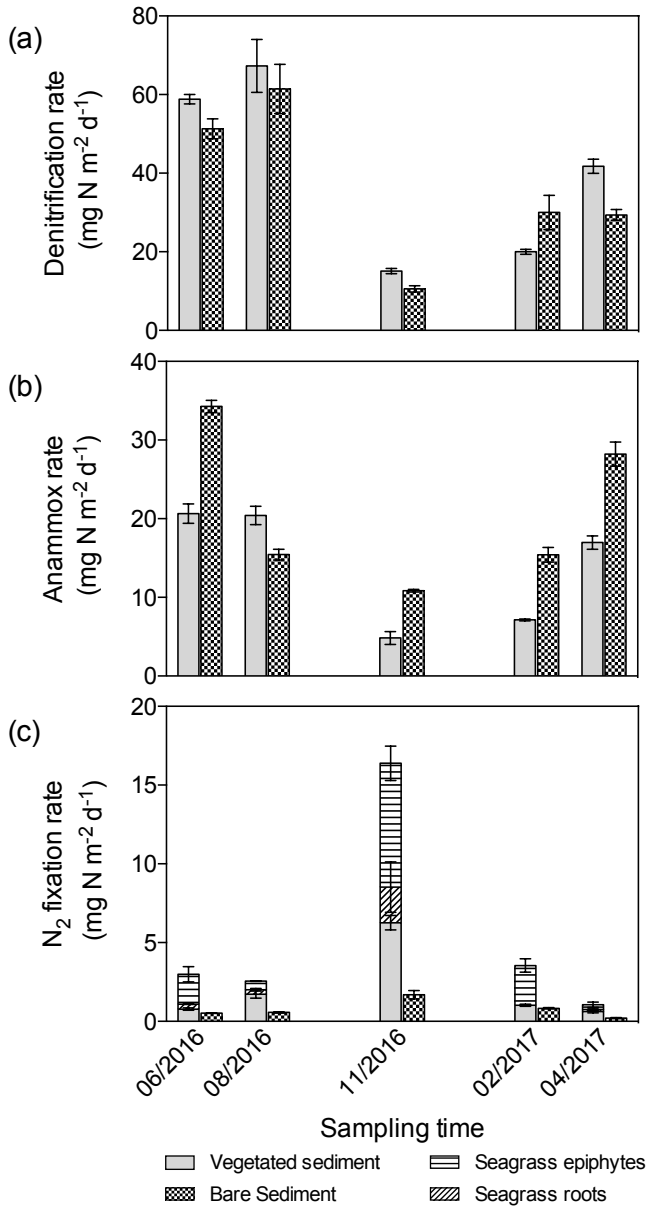
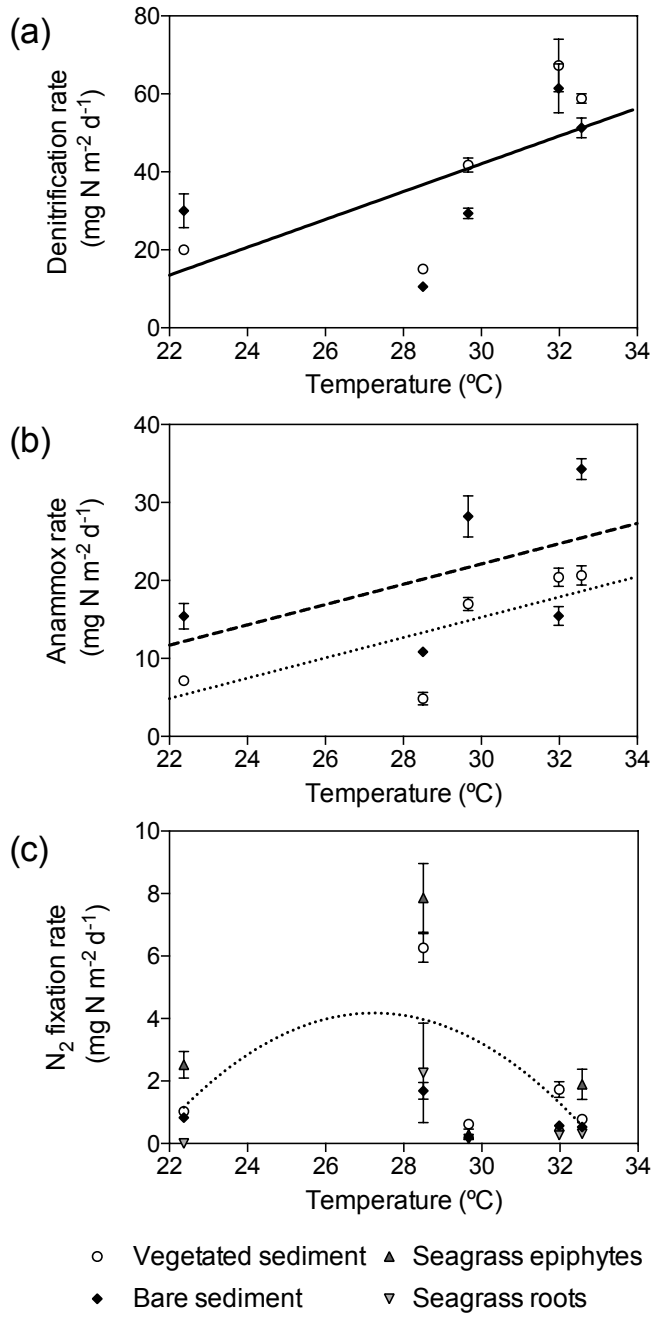


Fig. 4. Area integrated sediment rates along the year. **a**. Denitrification rates in vegetated (gray) and bare (square pattern) sediments. **b**. Anammox rates in vegetated (gray) and bare (square pattern) sediments. **c**. N_2 fixation rates in vegetated (gray) and bare (square pattern) sediments and in seagrass roots (angled stripes) and epiphytes (horizontal stripes). Error bars indicate SEM.

795 **Fig. 5**



796
797

798 **Fig. 5.** Relation of denitrification, anammox, and N_2 fixation rates with *in situ* seawater
799 temperature. **a**. Linear increase of denitrification rates (solid line) with temperature, showing
800 denitrification rates in vegetated sediments (white dots) and bare sediments (black diamonds). **b**.
801 Linear increase of anammox rates in vegetated (dotted line and white dots) and bare (dashed line
802 and black diamonds) sediments. **c**. Thermal response of N_2 fixation rates in vegetated sediments
803 (white dots), bare sediments (black diamonds), seagrass epiphytes (triangles) and roots (upside
804 down triangles), showing the fitted second-degree polynomial curve in vegetated sediment
805 (dotted line). Error bars indicate SEM.

Fig. 6

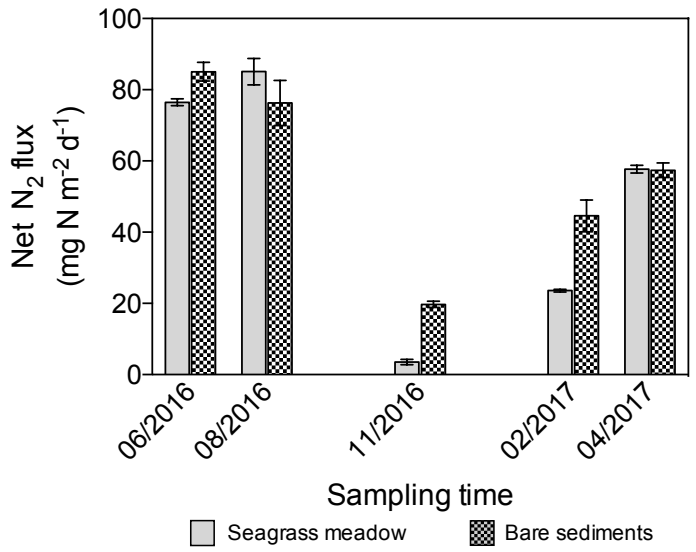
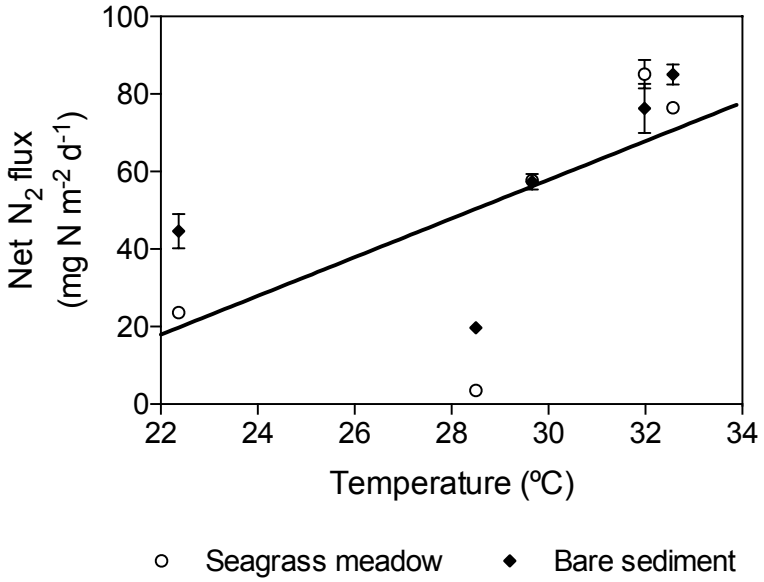


Fig. 6. Net N_2 flux in seagrass meadow (gray) and bare sediment (square pattern gray) along the year, considering sediment denitrification and anammox as N losses and sediment and seagrass roots and epiphytes N_2 fixation as new N inputs. Error bars indicate SEM.

813 **Fig. 7**



814
815
816 **Fig. 7.** Linear increase (solid line) of net N_2 fluxes in vegetated (white dots) and bare (black
817 diamonds) sediments. Error bars indicate SEM.
818

819 **Table 1.** Annual variation of *in situ* seawater temperature. Mean seawater temperature values are
820 daily averages of *in situ* seawater temperature and temperature range indicate daily oscillations
821 (minimum – maximum). Seawater temperature was recorded every 10 min during 24 h for each
822 sampling event.
823

Sampling time	Mean Seawater Temperature (°C)	Seawater Temperature Range (°C)
06/2016	32.5	31.6 – 33.6
08/2016	31.9	31.1 – 32.8
11/2016	28.4	27.7 – 29.2
02/2017	22.3	21.3 – 22.8
04/2017	29.5	28.7 – 30.0