



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

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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 (N₂) fixation has been reported to support seagrass growth. Therefore, the role of coastal marine systems dominated by seagrasses in the net N₂ flux remains unclear. Here, we measured denitrification, anaerobic ammonium oxidation (anammox), and N₂ fixation in a 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 (between 8 and 63-fold) than the N₂ 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 N₂ from sediments to the atmosphere. Despite the importance of this coastal lagoon in removing N from the system, N₂ fixation can contribute substantially to seagrass growth since N₂ 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 N₂ fixation increased with OM content. Denitrification and anammox increased linearly with temperature, while N₂ fixation showed a maximum at intermediate temperatures. Therefore, the forecasted warming could further increase the N₂ 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 (N₂) 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 N₂-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 N₂ (and N₂O partially), leading to the loss of fixed N. Globally, coastal denitrifica-

tion 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), N₂ 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 N₂, has also been regarded as an important process in marine sediments. It has been estimated to account for from nearly zero up to 80 % of the total N loss in sediments (Dalsgaard et al., 2005; Devol, 2015).

Within the coastal ocean, seagrass ecosystems support high rates of N₂ fixation (McGlathery, 2008), particularly in tropical and subtropical ecosystems (Welsh, 2000; Herbert, 1999). Nitrogen supplied by N₂ fixation can account for up to 90 % of plant nutrient requirements (Hansen et al., 2000). It has been suggested that seagrasses have a close mutualistic relationship with N₂-fixing bacteria inhabiting the rhizosphere (Herbert, 1999) and roots (Garcias-Bonet et al., 2016), where bacteria would benefit from root exudates and plants would benefit from fixed N supply. However, seagrass ecosystems also support high denitrification rates (Eyre et al., 2011b) and have been identified as key habitats for N loss in temperate (Eyre et al., 2016) and subtropical (Eyre et al., 2011a) estuaries. Although anammox rates have not been assessed for seagrass sediments as yet, Salk et al. (2017) recently reported very low anammox rates (0.18 μmol N m⁻² h⁻¹), despite accounting for 74 % of N loss, on bare sediment adjacent to a *Zostera muelleri* meadow in a sub-tropical estuarine system. Therefore, the role of seagrass ecosystems as net sinks or sources of N remains unclear. Welsh et al. (2000) reported very low denitrification rates compared to N₂ fixation rates in a temperate intertidal seagrass meadow, whereas denitrification seems to exceed N₂ fixation in tropical (Alongi et al., 2008) and sub-tropical (Eyre et al., 2011a) seagrass ecosystems. The balance between transformations of inert atmospheric N₂ and reactive N in seagrass ecosystems is an important driver of their net N budget (Hemminga et al., 1991), particularly in areas with limited N inputs from land. This is the case for seagrass meadows in arid regions lacking riverine inputs, such as the Red Sea, characterized by general oligotrophic conditions in surface waters (Raitzos et al., 2013; Weikert, 1987).

Nitrogen cycling in seagrass ecosystems is mediated by complex microbial communities requiring specific physicochemical conditions, which may ultimately determine the balance between transformations of inert atmospheric N₂ and reactive N. Seagrass meadows offer patchy sediment conditions, affected by the release of organic exudates and oxygen by seagrass roots (Pedersen et al., 1998), as well as the activity of burrowing infauna, which facilitates the exchange and diffusion of nutrients and allows the oxygenation of deep sediment layers (Welsh, 2003). These processes contribute to high spatial heterogeneity in seagrass sediments, therefore, modifying the redox potential (Enriquez et al., 2001) and al-

lowing for the co-occurrence of processes requiring different environmental conditions (Herbert, 1999; Hemminga et al., 1991).

Here, we test the following hypotheses: (i) that seagrasses and bare sediments in a coastal lagoon in the Red Sea are net N₂ sources and (ii) that the loss of reactive N from sediments to the atmosphere increases with temperature. Specifically, we assess the annual balance between losses of reactive N as N₂, via denitrification and anammox, and gains of reactive N, by N₂ fixation, in a tropical seagrass (*Enhalus acoroides*) meadow and the adjacent bare sediment in a coastal lagoon located in the central Red Sea. We first describe the environmental conditions in the sediments, based on microprofiles of oxygen (O₂), sulfide (H₂S), and redox, and then evaluate denitrification, anammox, and N₂ fixation rates in seagrass sediments and adjacent bare sediments. In addition, we analyze the thermal dependence of denitrification, anammox, and N₂ fixation throughout the annual in situ thermal range. The Red Sea is one of the warmest seas and is warming faster than other seas (Chaidez et al., 2017), thereby offering an opportunity to assess if the balance between losses of reactive N as N₂, via denitrification and anammox, and gains of reactive N by N₂ fixation, may be affected by warming.

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 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 (O₂, H₂S, and redox) and denitrification, anammox, and N₂ fixation rates across 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 h using an EXO1 Multiparameter Sonde (YSI, USA).

2.2 Sediment microprofiles

At each sampling event, we performed O₂, H₂S, 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 in 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 micropro-

file 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. (2015). Redox measurements were performed using Redox-200 microelectrodes (200 μm tip diameter). The electrode potential in the sediment was 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 h 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 with a batch incubation experiment with ^{15}N -labeled nitrogen compounds in vegetated sediment and the adjacent bare sediment. At each sampling event, we collected six cylindrical plastic cores (40 cm length and 5 cm in diameter, three 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. A ^{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 Dalsgaard, 2002). However, in this study, we used a simpler method developed previously (Amano et al., 2011; Yoshinaga et al., 2011). Briefly, about two grams of sediment was dispensed into 10 mL glass serum vials, which were sealed with butyl rubber stoppers and aluminum caps. All these procedures were performed in an anaerobic chamber (Coy Laboratory Products, Grass Lake Charter Township, MI), where O_2 concentration was < 1 ppm. Headspace in the vials was exchanged with highly pure He gas (> 99.9999 %) by vacuuming and purging. Positive pressure (50–75 kPa) was added to the headspace to prevent unintentional contamination with ambient air during the incubation and gas sampling. The vials containing oxygen-free sediment suspensions were pre-incubated overnight in the dark to elimi-

nate the remaining substrates. Four different combinations of ^{15}N labeled and/or unlabeled substrates were supplemented from anoxic stock solutions to these pre-incubated vials: (1) 0.5 mM $^{15}\text{NH}_4\text{Cl}$ (≥ 98 atom % ^{15}N , Sigma-Aldrich, Inc.); (2) 0.5 mM $^{15}\text{NH}_4\text{Cl}$ and 0.5 mM $\text{Na}^{14}\text{NO}_2$ (Sigma-Aldrich, Inc.); (3) 0.5 mM $\text{Na}^{15}\text{NO}_2$ (98 atom % ^{15}N , Sigma-Aldrich, Inc.); and (4) 0.5 mM K^{15}NO_3 (98 atom % ^{15}N , Sigma-Aldrich, Inc.). The concentration of the ^{15}N -labeled and unlabeled substrate was chosen as suggested previously (Amano et al., 2007). The vials were incubated under anoxic conditions at in situ temperature. The concentrations of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ gas were determined by gas chromatography-mass spectrometry (GC-MS) analysis at different intervals as described previously (Isobe et al., 2011a, b). In combination with GC inlet and peak integration software, a quadrupole MS system can be used to determine N_2 production rates < 10 $\text{nmol NL}^{-1} \text{d}^{-1}$ (Holtappels et al., 2011). A total of 50 μL of headspace gas were collected using a gas-tight syringe (VICI; Baton Rouge, LA, USA), immediately injected into a GC (Agilent 7890A system equipped with a CP-7348 PoraBond Q column) combined with 5975C quadrupole inert MS (Agilent Technologies; Santa Clara, CA, USA). The $m/z = 29$ and 30 values were monitored at the same retention time for each measurement. The amounts of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ gas were determined using a standard curve prepared with $^{30}\text{N}_2$ standard gas (> 98 % purity; Cambridge Isotope Laboratories; Tewksbury, MA, USA). The potential denitrification and anammox rates were estimated from the production of $^{29}\text{N}_2$ and $^{30}\text{N}_2$ using the equations (provided in Supplement) described previously (Holtappels et al., 2011; Yoshinaga et al., 2011).

All the batch tests were performed in triplicate. Finally, the denitrification and anammox rates were standardized to surface area integrating 3 cm sediment depth by averaging the rates measured at different horizons and taking into account the sediment bulk density.

2.4 Atmospheric N_2 fixation rates

We measured N_2 fixation by Acetylene Reduction Assay (Capone and Taylor, 1980) in seagrass roots, seagrass shoot epiphytes, vegetated sediment, and adjacent bare sediment. At each sampling time, we carefully collected shoots containing roots and placed them in zip-lock plastic bags. The shoots were immediately transported to the laboratory in a cooler box protected from sunlight. We also collected six cylindrical plastic cores (40 cm length and 10 cm in diameter, three replicate cores per each sediment type) containing at least 15 cm of undisturbed sediment. The sediment cores were transported immediately to the laboratory. Once in the laboratory the roots were carefully separated from shoots and rinsed with sterilized seawater. Then we placed 10 g (fresh weight) of healthy roots in a 250 mL glass bottle. Similarly, we placed one shoot (without roots) in a 500 mL glass bottle. We added 100 and 250 mL of fresh seawater collected

from the same location to the root and shoot samples, respectively, and the bottles were closed with lids fitted with gas-tight valves. In parallel, the sediment from the cores was extruded 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 $200 \mu\text{mol photons m}^{-2} \text{s}^{-1}$: 12 h dark) at in situ temperature.

We sampled the headspace five times, distributed across 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 ($60 \text{ m} \times 320 \mu\text{m} \times 1.5 \mu\text{m}$, 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 concentration in the equilibrated air according to Wilson et al. (2012) and applying the solubility coefficient of ethylene extracted from Breitbarth et al. (2004) as a function of temperature and salinity.

We run the following negative controls at each sampling event: (i) roots, shoots, and sediment without addition of acetylene-saturated seawater in order to confirm that ethylene was not naturally produced by our samples, and (ii) seawater collected from the study site and used in the preparation of the incubations with addition of acetylene-saturated seawater in order to measure the N_2 fixation due to pelagic diazotrophs. The ethylene production rate measured in the seawater control was subtracted from the ethylene production rates detected in our samples. The net ethylene rates (after subtracting the background seawater rate) were converted

into N_2 fixation rates by applying the common ratio of 3 mol of acetylene : 1 mol of N_2 (Welsh, 2000).

At the end of the incubation, we dried the roots, shoots, and sediment samples at 60°C and recorded the dry weight for further calculations. Moreover, we calculated the sediment organic matter (OM) content of each replicate sediment horizon by loss on ignition (Dean Jr., 1974). Then the sediment N_2 fixation rates were standardized to surface area integrated over 3 cm sediment depth by averaging the rates measured at the first 3 sediment horizons and taking into account the sediment bulk density in order to compare N_2 fixation to denitrification and anammox rates. The N_2 fixation rates of roots and shoot epiphytes were standardized to surface area taking into account the biomass density.

2.5 Statistical analysis

Differences in OM content (our continuous response variable) were tested considering the categorical explanatory variables “sediment type” (2 levels: vegetated and bare sediments) with the non-parametric Wilcoxon test. Furthermore, we analyzed OM content considering also as explanatory variable “sediment horizons” (4 levels: 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), and “sampling events” (5 levels: June, August, November, February, and April) by performing a generalized linear model (GLM) and considering their interaction. All the factors were fixed and orthogonal.

Differences in O_2 concentration and H_2S concentration between vegetated and bare sediments and between light and dark were tested by nonparametric Wilcoxon test.

Differences in denitrification, anammox, and N_2 fixation rates per gram of sediment along the sediment horizons between vegetated and bare sediments were tested by Wilcoxon matched-pairs signed rank test. Similarly, we test for differences between denitrification, anammox, and N_2 fixation rates by Wilcoxon matched-pairs signed rank test. Moreover, we analyzed the difference in denitrification, anammox, and N_2 fixation rates per gram of sediment considering the type of sediment and the sediment OM content as our categorical and continuous explanatory variables, respectively. Since the distribution of denitrification, anammox, and N_2 fixation rates per gram of sediment was not normal, we used a GLM to test for differences.

Finally, we analyzed the difference in depth-integrated denitrification, anammox, and N_2 fixation rates, our continuous response variables, considering the type of sediment and sampling event as our categorical explanatory variables. We furthermore analyzed the difference in depth-integrated denitrification, anammox, and N_2 fixation rates, considering the type of sediment and temperature as our categorical and continuous explanatory variables, respectively. Since the distribution of the depth-integrated denitrification and N_2 fixation rates was not normal, we used a GLM to test for differences, while we used a linear model test to analyze the depth-

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

Sampling time	Mean seawater temperature (°C)	Seawater temperature range (°C)
Jun 2016	32.5	31.6–33.6
Aug 2016	31.9	31.1–32.8
Nov 2016	28.4	27.7–29.2
Feb 2017	22.3	21.3–22.8
Apr 2017	29.5	28.7–30.0

integrated anammox rates as its distribution was normal. The effect of temperature and sediment type on the net N_2 flux was tested by using a linear model. All statistical analyses were performed using JMP (SAS Institute Inc., USA) and PRISM (GraphPad Software Inc., USA) statistical software.

3 Results

3.1 Water and sediment properties

The in situ daily average seawater temperature ranged from 22.3°C in February to 32.5°C in June (Table 1), while annual mean salinity was 41.2 ± 0.4 PSU. The OM content was consistently higher (about 40% higher) in the vegetated sediments compared to the bare sediment (nonparametric Wilcoxon test, $p < 0.0001$), with annual mean (\pm SEM) OM content of 13.5 ± 0.1 and 8.5 ± 0.1 % of sediment dry weight, respectively, and decreased with increasing depth (Fig. 1a). The sediment OM content significantly differed among sediment type, sampling event, and sediment horizon (GLM; sediment type \times sampling event \times sediment horizon $\chi^2_{12, 80} = 28.7$; $p = 0.004$). The maximum depth-integrated mean OM content in vegetated sediments (15% of sediment dry weight) and bare sediments (9.5% of sediment dry weight) was found in November and June, respectively.

Sediment O_2 microprofiles significantly differed between vegetated and bare sediments during light and dark measurements (nonparametric Wilcoxon test, $p = 0.0002$ and $p < 0.0001$, respectively) and between light and dark conditions in both vegetated and bare sediments (nonparametric Wilcoxon test, $p < 0.0001$ and $p < 0.0001$, respectively). The vegetated and bare sediments were anoxic below the sediment surface but the sediment depth at which anoxic conditions prevailed varied depending on sediment type, light or dark conditions, and the time of the year (Fig. 1b, c). In vegetated sediments, the anoxic conditions appeared at deeper layers than 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

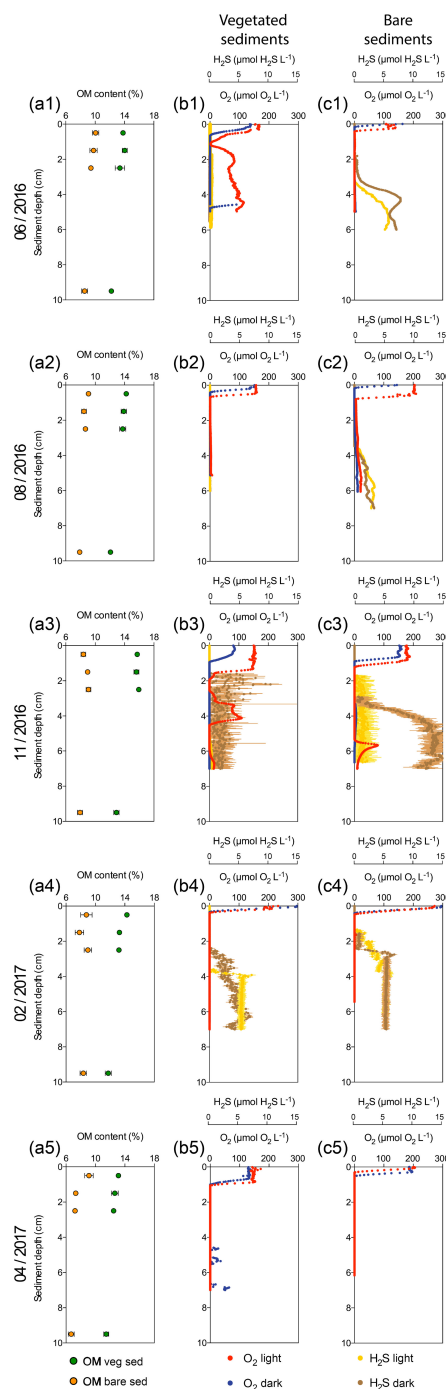


Figure 1. Characterization of *Enhalus acoroides* seagrass vegetated sediments and adjacent bare sediments at five sampling times throughout the year. (a1–a5) Sediment organic matter content in vegetated (green dots) and bare (orange dots) sediment horizons. (b1–b5) Vegetated sediment O_2 microprofiles 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–c5) 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).

average, the vegetated sediments were anoxic at 1 ± 0.3 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 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 across the year (Fig. 1b, c). Under light conditions, the H_2S concentration in bare sediments (median = $1.28 \mu\text{mol } H_2S \text{ L}^{-1}$) was significantly higher than in vegetated sediments (median = $0 \mu\text{mol } H_2S \text{ L}^{-1}$) (nonparametric Wilcoxon test, $p < 0.0001$). Similarly, under dark conditions, the H_2S concentration in bare sediments (median = $1.17 \mu\text{mol } H_2S \text{ L}^{-1}$) was significantly higher than in vegetated sediments (median = $0.008 \mu\text{mol } H_2S \text{ L}^{-1}$) (nonparametric Wilcoxon test, $p < 0.0001$). In vegetated sediments, the H_2S concentration was very low ($< 0.5 \mu\text{M}$) during the summer months (June and August, Fig. 1b1–b2) and the maximum H_2S concentration ($10.4 \mu\text{M}$) 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 ($15.2 \mu\text{M}$) 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 to -450 mV (Fig. S1 in the Supplement) 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–a2) for both vegetated and bare sediments. In vegetated sediments, the maximum denitrification rate ($20.52 \pm 0.6 \text{ nmol N g DW}^{-1} \text{ h}^{-1}$) was found in June and was almost twice the maximum rate measured in bare sediments ($11.5 \pm 4.2 \text{ nmol N g DW}^{-1} \text{ h}^{-1}$), which was found in August. Although the rates varied throughout the year, the maximum denitrification rates took place between 1 and 2 cm below the 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 ($6.88 \pm 0.5 \text{ nmol N g DW}^{-1} \text{ h}^{-1}$) was detected

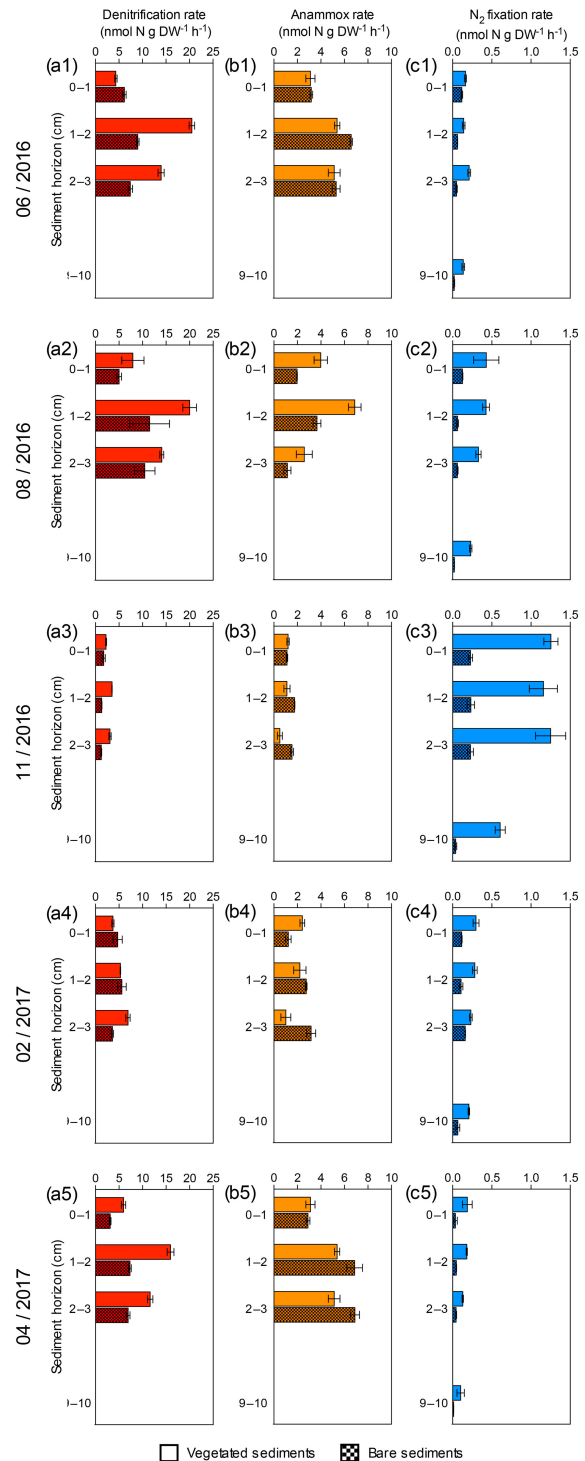


Figure 2. Sediment profiles of denitrification, anammox and N_2 fixation rates at five sampling times. (a1–a5) Sediment denitrification rates in vegetated (red) and bare (red square pattern) sediment horizons. (b1–b5) Sediment anammox rates in vegetated (orange) and bare (orange square pattern) sediment horizons. (c1–c5) Sediment N_2 fixation rates in vegetated (blue) and bare (blue square pattern) sediment horizons. Error bars indicate SEM.

in August and was similar to the maximum rate in bare sediments ($6.89 \pm 0.4 \text{ nmol N g DW}^{-1} \text{ h}^{-1}$), 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 ($1.25 \pm 0.1 \text{ nmol N g DW}^{-1} \text{ h}^{-1}$) 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 \times 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 observed 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 \times 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 \times 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.

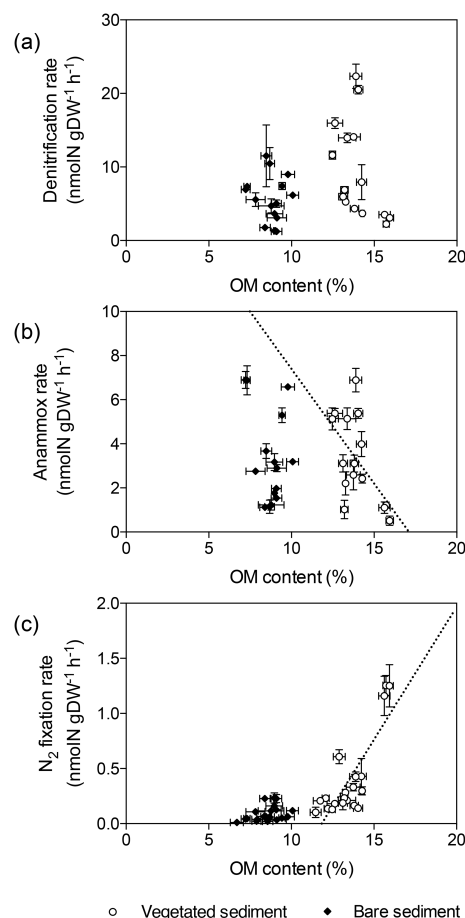


Figure 3. Relation of denitrification, anammox, and N_2 fixation rates with sediment OM content. (a) Denitrification rates in vegetated sediments (white dots) and bare sediments (black diamonds). (b) Anammox rates in vegetated (white dots) and bare (black diamonds) sediments, showing the linear decrease of anammox rates in vegetated sediments with increasing OM content (dotted line) (c) N_2 fixation rates in vegetated sediments (white dots) and bare sediments (black diamonds), showing the linear increase of N_2 fixation rates in vegetated sediments with increasing OM content (dotted line). Error bars indicate SEM.

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

Temperature had a significant effect on depth-integrated denitrification rates regardless of the type of sediment (GLM; temperature $\chi^2_{1, 27} = 16.67$, $p < 0.0001$; sediment type $\chi^2_{1, 27} = 0.53$, $p = 0.46$). Depth-integrated denitrification rates increased linearly with temperature ($Y = 3.569X - 65$, Fig. 5a). Temperature and sediment type had a significant effect on depth-integrated anammox rates (lm; temperature $F_{1, 29} = 14.8$, $p = 0.0007$; sediment type, $F_{1, 29} = 7.7$, $p = 0.01$), with rates increasing linearly in vegetated ($Y = 1.3X - 20.36$) and bare ($Y = 1.3X - 16.94$) sediments (Fig. 5b). However, depth-integrated N_2 fixation rates did

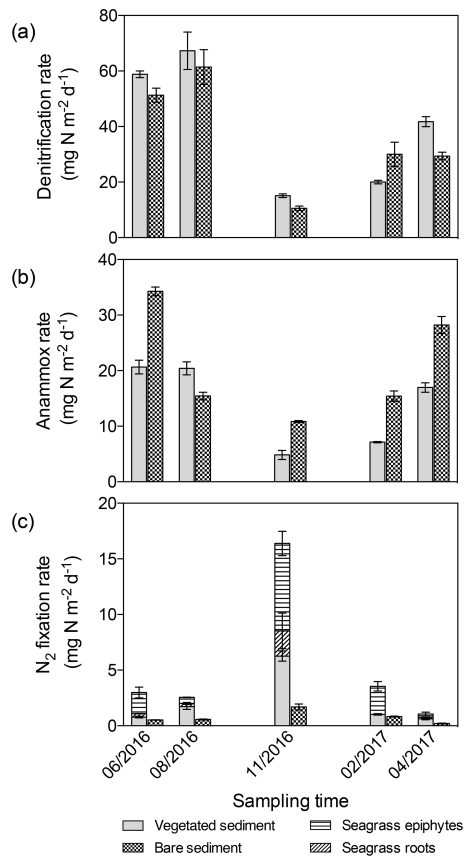


Figure 4. Area integrated sediment rates throughout the year. **(a)** Denitrification rates in vegetated (gray) and bare (square pattern) sediments. **(b)** Anammox rates in vegetated (gray) and bare (square pattern) sediments. **(c)** N₂ fixation rates in vegetated (gray) and bare (square pattern) sediments, in seagrass roots (angled stripes), and epiphytes (horizontal stripes). Error bars indicate SEM.

not increase linearly with temperature and the differences in rates were explained by sediment type (GLM; sediment type $\chi^2_{1, 27} = 4.93$, $p = 0.03$). Sediment N₂ fixation rates in vegetated and bare sediments showed a different thermal response than denitrification and anammox processes, with maximum rates reported at 28.5 °C and decreasing rates at either lower and higher temperatures (Fig. 5c). N₂ fixation rates followed a second-degree polynomial curve ($Y = 16.94 - 0.45X - 0.13X^2$, $r^2 = 0.40$, $p < 0.05$) in vegetated sediments. N₂ fixation rates in seagrass roots and epiphytes showed the same annual pattern that the rates reported for the rhizosphere. The maximum rates in seagrass roots ($21.9 \pm 210.7 \mu\text{g N g DW}^{-1} \text{d}^{-1}$) and epiphytes ($10.4 \pm 1.5 \mu\text{g N g DW}^{-1} \text{d}^{-1}$) were also recorded in November when in situ seawater temperature was 28.5 °C (Fig. 5c).

3.4 Net N₂ fluxes

The net N₂ fluxes ranged from 3.6 ± 0.8 and $19.73 \pm 0.9 \text{ mg N m}^{-2} \text{d}^{-1}$ in November, to 85.1 ± 3.7 and $85.1 \pm$

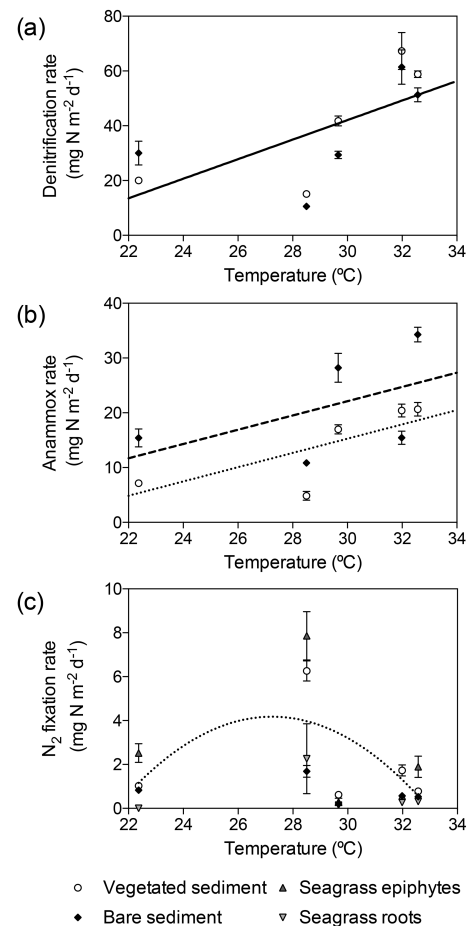


Figure 5. Relation of denitrification, anammox, and N₂ fixation rates to in situ seawater temperature. **(a)** Linear increase of denitrification rates (solid line) with temperature, showing denitrification rates in vegetated sediments (white dots) and bare sediments (black diamonds). **(b)** Linear increase of anammox rates in vegetated (dotted line and white dots) and bare (dashed line and black diamonds) sediments. **(c)** Thermal response of N₂ fixation rates in vegetated sediments (white dots), bare sediments (black diamonds), seagrass epiphytes (triangles) and roots (upside down triangles), showing the fitted second-degree polynomial curve in vegetated sediment (dotted line). Error bars indicate SEM.

$2.6 \text{ mg N m}^{-2} \text{d}^{-1}$ in summer months for the seagrass meadow and bare sediments, respectively (Fig. 6). The net N₂ flux significantly differed among sampling events but not between sediment type (1m; sampling event $F_{4, 9} = 24.76$, $p = 0.004$; sediment type, $F_{1, 9} = 1.83$, $p = 0.25$). Net N₂ flux increased linearly with temperature ($Y = 4.99X - 91.86$, $r^2 = 0.43$, $p < 0.05$, Fig. 7).

4 Discussion

The sediment organic matter content in the Red Sea lagoon system studied here was extremely high, especially in the

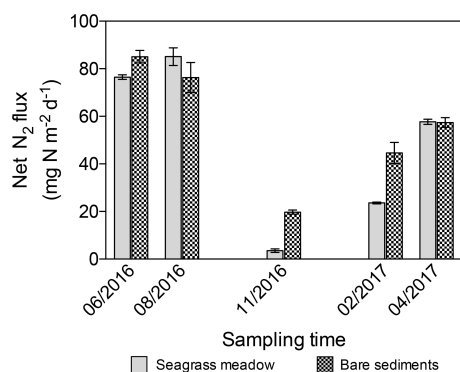


Figure 6. Net N₂ flux in seagrass meadow (gray) and bare sediment (square pattern gray) throughout the year, considering sediment denitrification and anammox as N losses and sediment and seagrass roots and epiphytes N₂ fixation as new N inputs. Error bars indicate SEM.

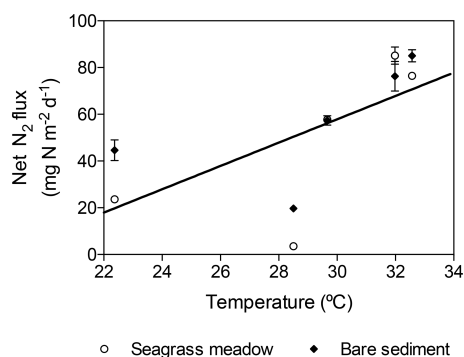


Figure 7. Linear increase (solid line) of net N₂ fluxes in vegetated (white dots) and bare (black diamonds) sediments. Error bars indicate SEM.

vegetated sediments (mean = 13.5 %), compared to the values reported for other seagrass sediments (mean = 4.1 %, Kennedy et al., 2010). The higher sediment organic matter content in vegetated sediments, compared to bare sediments, corroborates the evidence that seagrasses accumulate and store large amounts of organic carbon in their sediments (Duarte et al., 2005). In addition, seagrasses modified the physicochemical conditions of the sediment (Enriquez et al., 2001), as reflected in higher O₂ and lower sulfide sediment concentrations than those in the adjacent bare sediment. Moreover, O₂ profiles showed higher variability in vegetated sediments. This can be directly related to bioturbation (Kristensen et al., 2012) and to the radial oxygen loss by roots leading to oxic layers at depth (Pedersen et al., 1998), enhancing the complexity and heterogeneity of seagrass sediments.

The denitrification rate in *E. acoroides* sediments reported here (annual mean = 34.9 ± 10.3 mg N m⁻² d⁻¹) is a 6-fold increase over the rate reported for a restored *Zostera marina* meadow in Virginia using an in situ push-pull incubation

method (Aoki and McGlathery, 2017), a 1.3 to 2.5-fold increase over the rate previously reported for tropical meadows dominated by *E. acoroides* on slurries from the top 5 cm sediment (Alongi et al., 2008), comparable to the rates reported for temperate seagrasses (Eyre et al., 2016), and 8-fold lower than the rates reported for sub-tropical estuarine seagrasses (Eyre et al., 2011a) using in situ benthic chambers. However, the use of the ¹⁵N isotope pairing technique on sediment slurries could have underestimated denitrification rates reported here. Measuring N₂ fluxes on intact sediment cores has been proved to better account for coupled nitrification and denitrification than the ¹⁵N isotope pairing technique (van Luijn et al., 1996). In addition, sediment slurries disturb the sediment structure and redox gradient, and, therefore, might prevent the coupled nitrification and denitrification in the transition layers from oxic to anoxic conditions (Eyre et al., 2002; Herbert, 1999). Since the coupled nitrification and denitrification has been reported to be important in continental shelf and coastal sediments (Herbert, 1999; Gardner and McCarthy, 2009; Christensen et al., 1987); the denitrification rates in this coastal lagoon could be higher than actual reported values.

Overall, the observed denitrification rates were higher in vegetated sediments than bare sediments when expressed per gram of dried sediment. However, we did not find differences between depth-integrated denitrification rates in vegetated and bare sediments (annual mean = 34.9 ± 10.3 and 31.6 ± 8.9 mg N m⁻² d⁻¹, respectively) contrary to previous findings (Eyre et al., 2011b).

The potential sediment anammox rates reported here, ranging from 0.5 to 6.9 nmol N g DW⁻¹ h⁻¹, are comparable to potential anammox rates reported elsewhere (i.e., from 0 to 8 nmol N cm⁻³ h⁻¹ in subtropical mangrove sediments (Meyer et al., 2005); from < 0.5 to 7.6 nmol N g DW⁻¹ h⁻¹ in marine sediments from a fjord in Sweden (Brandsma et al., 2011) and from 0 to 3 nmol N cm⁻³ h⁻¹ in sandy sediments in the southern North Sea; Bale et al., 2014). However, the potential sediment anammox rates detected here (annual mean depth-integrated anammox rates = 12.4 ± 3.4 and 19.8 ± 4.4 mg N m⁻² d⁻¹ in vegetated and bare sediments, respectively) are higher than the only estimate available, to the best of our knowledge, for a seagrass ecosystem (0.06 mg N m⁻² d⁻¹ in bare sediment adjacent to a subtropical seagrass meadow in Australia on intact cores; 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. (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 N₂ 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 N₂ fixation rates in sediments of a tropical mixed meadow dominated by *E. acoroides*

($19.4 \pm 3.2 \text{ mg N m}^{-2} \text{ d}^{-1}$, Alongi et al., 2008). Similarly, Moriarty and O'Donohue (1993) reported higher N_2 fixation rates for a mixed meadow dominated by *E. acoroides* ($25 \pm 2.6 \text{ mg N m}^{-2} \text{ d}^{-1}$) than those reported here during the same time of the year ($16.4 \pm 0.4 \text{ mg N m}^{-2} \text{ d}^{-1}$), although with a smaller contribution from leaf epiphytes ($4.2 \pm 0.1 \text{ mg N m}^{-2} \text{ d}^{-1}$) compared with our N_2 fixation rates from epiphytes ($7.9 \pm 1.1 \text{ mg N m}^{-2} \text{ d}^{-1}$). 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, 2016). Moreover, the N_2 fixation rates previously reported for surface-sterilized *E. acoroides* roots ($0.13 \text{ mg N m}^{-2} \text{ d}^{-1}$; Raja et al., 2012) are 17-fold lower than the rates reported here ($2.3 \pm 1.5 \text{ mg N m}^{-2} \text{ d}^{-1}$) for the same seawater temperature (29°C), pointing out 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 $15.2 \mu\text{mol H}_2\text{S L}^{-1}$ 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 $10 \mu\text{mol H}_2\text{S L}^{-1}$ for seagrass decline (Calleja et al., 2007).

The contrasting annual patterns in denitrification and anammox compared to those of N_2 fixation, with the 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 (29°C) 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 35°C 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 (Enríguez et al., 1993). OM from phytoplankton decomposes faster than OM from seagrasses, due to their higher N content and therefore lower C : N : P ratios. Eyre et al. (2013) demonstrated that the source of the OM, and therefore, its C : N ratio controls denitrification rates in coastal sediments. Tibbles et al. (1994) showed an increase in sediment N_2 fixation following the addition of complex plant polysaccharides and Fulweiler et al. (2013) argued that an increase in the C : N ratio of OM was responsible for the decrease in denitrification and the increase in N_2 fixation, in agreement with the effect of OM reported here.

The net N_2 fluxes in the Red Sea lagoon ecosystem indicates this ecosystem supports a net loss of reactive N as N_2 gas throughout the year, as sediment denitrification and anammox consistently exceeded N_2 fixation in sediment and seagrass tissues. Alongi et al. (2008) also reported higher denitrification than N_2 fixation rates in an *E. acoroides* meadow. Integrating the average seasonal rates, we estimate the annual N loss in $14.9 \text{ g N m}^{-2} \text{ yr}^{-1}$ in the seagrass meadow and $18.2 \text{ g N m}^{-2} \text{ yr}^{-1}$ in bare sediments. Despite the lack of rivers discharging into the Red Sea, the occasional heavy rains, groundwater discharge, and atmospheric deposition might lead to high reactive N loads reaching coastal systems (Voss et al., 2013). Therefore, the high denitrification and anammox rates in this coastal lagoon provide a mechanism adding resistance to excess N inputs (Zarnoch et al., 2017; Galloway et al., 2003), which might lead to permanent seagrass losses due to hypoxia after algal blooms produced by severe eutrophication (Herbert, 1999; Duarte, 1995). However, dissimilatory nitrate reduction to ammonium (DNRA) competes with denitrification by reducing nitrate availability. In a shallow estuary, DNRA was identified as an important nitrate loss pathway, with rates comparable to denitrification rates (An and Gardner, 2002); and in a restored *Zostera marina* meadow, DNRA accounted for 45 % of sediment nitrate reduction (Aoki and McGlathery, 2017). Therefore, the net N loss reported here 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 ($\delta^{15}\text{N}_{\text{leaves}} = 0.17\text{‰}$ and $\delta^{15}\text{N}_{\text{rhizomes}} = -1.56\text{‰}$;

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 N₂ fixation, which showed a maximum at about 28 °C, leads to an increase in the net N₂ flux with temperature (Fig. 6). This has important implications in a context of the rapid warming of the Red Sea (Chaidez et al., 2017). In particular, a further increase in temperature, with maximum temperatures in excess of 33 °C 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 thus on seagrass productivity and their capacity to mitigate climate change.

5 Conclusion

The studied coastal lagoon ecosystem supported a net loss of reactive N as N₂, with anammox accounting for about one-third of N₂ production. However, N₂ 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 the already warm Red Sea.

Data availability. Underlying data are provided in the Supplement.

Supplement. The supplement related to this article is available online at: <https://doi.org/10.5194/bg-15-7333-2018-supplement>.

Author contributions. NGB and CMD designed the study. NGB and MF performed the fieldwork. NGB performed the N₂ fixation measurements. MF performed the sediment microprofiles. MA and DRS performed the denitrification and anammox activity measurements. NGB, MF and CMD interpreted the results. NGB wrote the first draft of the paper. All authors contributed substantially to the final paper.

Competing interests. The authors declare that they have no conflict of interest.

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