



# Seasonal, daily and diel N<sub>2</sub> effluxes in permeable carbonate sediments

B. D. Eyre, I. R. Santos, and D. T. Maher

Centre for Coastal Biogeochemistry, Southern Cross University, P.O. Box 157, Lismore, NSW 2480, Australia

Correspondence to: B. D. Eyre (bradley.eyre@scu.edu.au)

Received: 2 November 2012 – Published in Biogeosciences Discuss.: 10 December 2012

Revised: 14 March 2013 – Accepted: 25 March 2013 – Published: 18 April 2013

**Abstract.** Benthic metabolism and inorganic nitrogen and N<sub>2</sub> flux rates (denitrification) were measured in permeable carbonate sands from Heron Island (Great Barrier Reef). Some of the N<sub>2</sub> flux rates were among the highest measured in sediments. All benthic fluxes showed a significant difference between seasons with higher rates in summer and late summer. There was no distinct response of the benthic system to mass coral spawning. Instead, changes in benthic fluxes over 12 days in summer appear to be driven by tidal changes in water depth and associated changes in photosynthetically active radiation reaching the sediments. Dark N<sub>2</sub> fluxes were strongly correlated to benthic oxygen consumption across all sites and seasons ( $r^2 = 0.63$ ;  $p < 0.005$ ; slope = 0.035). However, there were seasonal differences with a steeper slope in summer than winter, reflecting either more efficient coupling between respiration and nitrification–denitrification at higher temperatures or different sources of organic matter. Adding data from published studies on carbonate sands revealed two slopes in the dark N<sub>2</sub> flux versus benthic oxygen consumption relationship. The lower slope (0.035) was most likely due to high carbon:nitrogen (C:N) organic matter from coral reefs, and associated assimilation of nitrogen by heterotrophic bacteria including enhanced heterotrophic N-fixation, but competition by benthic microalgae or inefficient coupling between respiration and nitrification–denitrification cannot be excluded. The steeper slope (0.089) was most likely due to respiration being driven by low C:N phytodetritus. If the different slopes were driven by the sources of organic matter, then global estimates of continental shelf denitrification are probably about right. In contrast, global estimates of continental shelf denitrification may be over-estimated if the low slope was due to inefficient coupling between respiration and

nitrification–denitrification and also due to reduced N<sub>2</sub> effluxes in the light associated with competition by benthic microalgae for nitrogen and N-fixation.

## 1 Introduction

Denitrification permanently removes nitrogen from an ecosystem by converting fixed nitrogen to dinitrogen gas, which can then be lost to the atmosphere. The importance of denitrification, because it may control system production by removing nitrogen that would otherwise be available for primary producers, is demonstrated by the numerous denitrification studies in most types of ecosystems (Seitzinger et al., 2006). Continental shelves account for a third to half of global denitrification (Middelburg et al., 1996; Seitzinger et al., 2006). However, these estimates are based mostly on measurements and calculations from muddy sediments, and recent modelling of permeable quartz sands suggest that global rates of continental shelf denitrification are over-estimated by a factor of 2 to 7 (Cardenas et al., 2008). In contrast, Gao et al. (2012) argue that global rates of continental shelf denitrification may be under-estimated due to high rates of denitrification measured in permeable quartz sands.

Permeable carbonate sands cover large areas of coral reefs, and overall around 40% of continental shelves (Milliman and Droxler, 1996), but there have been few studies of denitrification in this type of sediment. Of the few measurements of denitrification in carbonate sediments, all are in coral reef sediments, and all earlier studies were done using the C<sub>2</sub>H<sub>2</sub> blockage technique on ex situ disturbed slurries (Seitzinger and D'Elia, 1985; Capone et al., 1992; Miyajima et al. 2001). The low dark denitrification rates (0.2

to 19  $\mu\text{mol N}_2\text{-N m}^{-2}\text{ h}^{-1}$ ) most likely reflects the C<sub>2</sub>H<sub>2</sub> blockage technique underestimating coupled nitrification–denitrification due to inhibition of nitrification (Hynes and Knowles, 1978). More recently higher direct N<sub>2</sub> fluxes (3 to 94  $\mu\text{mol N}_2\text{-N m}^{-2}\text{ h}^{-1}$ ) have been measured in the dark using the background removal technique (Nowicki, 1994) in fine to medium carbonate sands at inter-reef sites on the northern and central Great Barrier Reef (GBR) (Alongi et al., 2008) and in very coarse carbonate sands in the southern GBR (Alongi et al., 2011).

Only one in situ denitrification study has tried to assess the effect of advective pore water flow on rates in permeable carbonate sediments (Eyre et al., 2008). As the advective flow in benthic chambers increased, the N<sub>2</sub> fluxes changed from a diffusive uptake to an increasing efflux (Eyre et al., 2008). Stimulation of denitrification by advection has also been seen in several studies in permeable quartz sediments (Cook et al. 2006; Rao et al., 2007; Gihring et al., 2010; Gao et al., 2012). The quantity and quality (source) of organic matter supply to carbonate sediments was also an important driver of denitrification with a rapid increase in N<sub>2</sub> effluxes in response to the episodic deposition of phytodetritus associated with coral mass spawning (Eyre et al., 2008). Measured denitrification rates with advection were also much higher (34 to 480  $\mu\text{mol N}_2\text{-N m}^{-2}\text{ h}^{-1}$ ) than previously measured rates in carbonate sediments without advection (Eyre et al., 2008). However, the study of Eyre et al. (2008) was limited to dark denitrification rates measured during only one sampling season (early summer) and in only one sediment grain size. The hypothesis of this current study is that denitrification rates in permeable carbonate sands will vary spatially and temporally in response to controlling factors such as carbon and nitrogen supply and temperature. As such, denitrification rates were measured over a diel cycle in very coarse and coarse permeable carbonate sands on seasonal (summer, late summer, winter, spring), daily (12 consecutive days in summer) and episodic (following a coral-spawning event) time scales. Benthic metabolism and inorganic nitrogen fluxes were also measured to elucidate the factors controlling N<sub>2</sub> fluxes (denitrification). The implications of this work for coral reef and global nitrogen budgets will also be discussed.

## 2 Methods

### 2.1 Study site

Sampling was undertaken on the Heron Island reef flat at the southern end of the Great Barrier Reef about 70 km off the coast of Gladstone, Australia (23°26′24″ S, 151°54′36″ E). The reef flat is a mosaic of different coral species and carbonate sands. Overall 85 % of the reef lagoon at Heron Island consists of sand (Glud et al., 2008). The very coarse sand site was about 50 m from the eastern Research Beach shoreline near the site of Glud et al. (2008) and Eyre et al. (2008);

the coarse sand site was in Shark Bay at the southern end of the island near the site of Wild et al. (2004). Both the coarse and very coarse sediments contain < 1 % of silt and clay and have a low organic carbon (< 0.24 %) and nitrogen content (0.042 %)(Wild et al., 2004; Eyre et al., 2008). The coarse sands (Wentworth scale) had an average grain size of 536 microns, a permeability of  $2.1 \times 10^{-10}\text{ m}^2$  and a porosity of 44 %; the very coarse sands had an average grainsize of 1.160 microns, a permeability of  $0.6 \times 10^{-10}\text{ m}^2$  and a porosity of 46 % (Santos et al., 2012a). Average daily water temperature at the study site varied over the study period from 19.1 °C in winter (August) to 27.9 °C in summer (February).

### 2.2 Chamber measurements and sample analysis

A summary of the benthic chamber sampling campaigns is given in Table 1. Round benthic chambers with an internal diameter (i.d.) of 190 mm and a height of 330 mm (Huetel and Gust, 1992) were inserted into the permeable sands between outcrops of coral to retain a water column height of 190 to 240 mm. The chambers were deployed between 17.00 and 18.00 h on each day and left uncapped for about 3 h to equilibrate. The chambers were stirred at 40 RPM, which induced a sediment percolation rate of approximately  $43\text{ L m}^{-2}\text{ d}^{-1}$  (Glud et al., 2008). Although this is at the low range of advective rates estimated for permeable sediments in the Heron Island reef lagoon (0 to  $600\text{ L m}^{-2}\text{ d}^{-1}$ ; average  $150\text{ L m}^{-2}\text{ d}^{-1}$ ; Santos et al., 2010), these higher rates include all advective processes (e.g. tidal pumping), not just the small-scale advective processes in the sediments (e.g. topography induced advection). Most importantly, it was the same stirring rate that was used for most of the benthic metabolism and denitrification measurements from previous studies at the same site (i.e. Glud et al., 2008; Eyre et al., 2008), allowing a direct comparison of measured rates. A range of stirring rates was not used as the effect of different advection rates on benthic metabolism and denitrification in permeable carbonate sands has been well studied (Wild et al., 2004; Glud et al., 2008; Eyre et al., 2008; Santos et al., 2012a).

During the summer incubations a HOBO logger was placed on the lid of one chamber, and photosynthetically active radiation (PAR) was measured every 30 min and water depth every hour. HOBO Lux units were converted to  $\mu\text{E m}^{-2}\text{ s}^{-1}$  by multiplying by 0.0165 and then calibrated against a Li-Cor-192 light meter. All the benthic incubations were undertaken over a 20.00 h diel cycle. The chambers were then capped at 21.00 h, and the first samples from the chambers were collected. Subsequent samples were collected at 2.00 h, 5.00 h (dawn), 12.00 h and 17.00 h (dusk). N<sub>2</sub> : Ar samples were only collected at 21.00 h, 2.00 h, 5.00 h and 8.00 h, as the formation of bubbles during daytime confounded all gas samples collected after 8.00 h. Dissolved oxygen concentrations in the 40 RPM chambers typically closely follow dissolved oxygen variations in the water column (Glud et al., 2008; Cyronak et al., 2013). During the

**Table 1.** Summary of benthic chamber sampling campaigns.

Sample Campaign	Sediment Type	Chamber Incubation Length	Chamber Replication
Winter (Aug 2009)	Very coarse and coarse sands	One 21-h diurnal cycle	Triplicate
Summer (Dec 2009)	Very coarse sands	Twelve consecutive 21-h diurnal cycles	Triplicate on days 1 to 7 Single on days 8 to 12
Late summer (Feb 2010)	Very coarse and coarse sands	One 21-h diurnal cycle	Duplicate
Spring (Oct 2010)	Very coarse sands	One 21-h diurnal cycle	Duplicate

summer campaign the chambers were moved between 17.00 and 18.00 h within a 100 m<sup>2</sup> area and again left uncapped for 3.00 h to equilibrate before re-starting the sampling cycle.

On each sampling occasion two 50 mL pre-soaked (in site water) polypropylene syringes of water were collected from each chamber, and as a sample was withdrawn an equal amount was replaced from the bottom water. Within 10 min of sampling, 20 mL of sample was transferred to a polypropylene vial for dissolved oxygen measurements and 30 mL of sample was immediately filtered through a 0.45 µm cellulose acetate membrane filter (Sartorius) into two 10 mL acid- and sample-rinsed polypropylene vials. To minimise the introduction of bubbles, N<sub>2</sub>: Ar samples were collected in triplicate 7 mL gas-tight glass vials with glass stoppers, by adding a piece of gas-tight tubing to the syringe and filling from the bottom of the vial until it overflowed (Eyre et al., 2008). Two vials for nutrient samples were immediately frozen at -20 °C. N<sub>2</sub>: Ar samples were poisoned with 20 µL of 5% HgCl<sub>2</sub> and stored submerged at ambient temperature. Details of the nutrient and N<sub>2</sub>: Ar analysis are given in Eyre and Ferguson (2005). Dissolved oxygen measurements ( $\pm 0.01$  mg L<sup>-1</sup>) were undertaken using a HACH LDO HQ-10.

### 2.3 Benthic flux calculations

Fluxes across the sediment–water interface were calculated using the start and end point concentration data, corrected for the addition of replacement water, as a function of incubation time, core water volume, and surface area. Dark flux rates were calculated using concentration data from 21.00 h to 5.00 h; light flux rates were calculated using concentration data from 5.00 to 17.00 h. N<sub>2</sub> light flux rates were calculated using concentration data from 5.00 to 8.00 h:

$$- \text{net flux rates} = ((\text{hourly dark rates} \times \text{hours of darkness}) + (\text{hourly light rates} \times \text{hours of daylight}))/24 \text{ h};$$

- gross benthic oxygen production (GPP) = light O<sub>2</sub> flux (NPP) – dark O<sub>2</sub> flux (respiration);
- benthic O<sub>2</sub> *p/r* = GPP × daylight hours/respiration × 24 h;
- and it was assumed that the photosynthetic quotient (PQ) and respiratory quotient (RQ) = 1.

Careful consideration was given to the possible effect of bubbles on N<sub>2</sub> fluxes (Eyre et al., 2002). All samples over 96 % O<sub>2</sub> saturated were excluded from N<sub>2</sub> flux calculations because it was clear that these samples were affected by bubbles. As such, many light rates are missing due to samples being over 96 % O<sub>2</sub> saturated by 8.00 h. In some cases the water column was still saturated (> 96 %) when the first dark sample was taken (21.00 h); as such, some dark N<sub>2</sub> rates were calculated using concentration data from 2.00 to 5.00 h. Bubbles have the effect of reducing the first dark sample N<sub>2</sub> concentration, giving much higher dark N<sub>2</sub> flux rates, and reducing the last light sample N<sub>2</sub> concentration, giving lower light N<sub>2</sub> flux rates or apparent rates of N-fixation. Many net N<sub>2</sub> fluxes are also missing as they could only be calculated when light fluxes were available. Because N<sub>2</sub> fluxes were measured, they include both canonical denitrification and anammox. The terms N<sub>2</sub> efflux and denitrification are used interchangeably.

### 2.4 Statistical Analysis

To determine the influence of sediment type (very coarse, coarse) and season (winter, spring, summer, late summer) on benthic fluxes, a fixed-effects two-level linear mixed model (LMM) was used. The two-level model incorporated sediment type and season as fixed effects. As intra-class correlation is likely within the fixed effects, the LMM procedure was preferred over the commonly used general linear model (GLM) and standard one-way ANOVA models. LMM does not assume independent observations, and it allows greater flexibility in fitting and testing heterogeneous covariance

**Table 2.** Results from linear mixed model analysis of the effect of season and sediment type on benthic fluxes.

	Season	Sediment Type	Season × Sediment Type
Dark NH <sub>4</sub> <sup>+</sup>	$F = 4.593_{3,62} p = 0.006$ (Summer < winter, late summer, spring)	$F = 0.011_{1,62} p = 0.911$	$F = 0.079_{1,62} p = 0.780$
Light NH <sub>4</sub> <sup>+</sup>	$F = 1.558_{3,63} p = 0.208$	$F = 0.730_{1,63} p = 0.396$	$F = 0.707_{1,63} p = 0.404$
Net NH <sub>4</sub> <sup>+</sup>	$F = 6.996_{3,62} p < 0.001$ (Summer < winter, late summer, spring)	$F = 0.366_{1,62} p = 0.547$	$F = 1.606_{1,62} p = 0.210$
Dark NO <sub>3</sub> <sup>-</sup>	$F = 39.202_{3,62} p < 0.001$ (Summer < late, summer < spring, winter)	$F = 10.654_{1,62} p = 0.002$ (Coarse < very coarse)	$F = 4.296_{1,62} p = 0.042$ (Late summer coarse < very coarse)
Light NO <sub>3</sub> <sup>-</sup>	$F = 4.040_{3,64} p = 0.011$ (Spring < winter)	$F = 10.293_{1,64} p = 0.002$ (Coarse < very coarse)	$F = 1.000_{1,64} p = 0.321$
Net NO <sub>3</sub> <sup>-</sup>	$F = 20.076_{3,64} p < 0.001$ (Summer < Late Summer, Spring < Winter)	$F = 20.540_{1,64} p < 0.001$ (Coarse < Very coarse)	$F = 2.792_{1,64} p = 0.100$
Dark DO (respiration)	$F = 18.408_{3,62} p < 0.001$ (Summer, late Summer < spring, winter)	$F = 0.005_{1,62} p = 0.944$	$F = 9.922_{1,62} p = 0.003$ (Winter very coarse < coarse; late summer coarse < very coarse)
Light DO (NPP)	$F = 68.988_{3,63} p < 0.001$ (Spring < winter < summer, late summer)	$F = 0.444_{1,63} p = 0.508$	$F = 3.917_{1,63} p = 0.052$
GPP	$F = 57.030_{3,63} p < 0.001$ (Spring, winter < summer, late summer)	$F = 0.629_{1,63} p = 0.431$	$F = 5.373_{1,63} p < 0.024$ (Winter very coarse < coarse; late summer coarse < very coarse)
P/R DO	$F = 22.276_{3,62} p < 0.001$ (Spring, winter < summer, late summer)	$F = 2.042_{1,62} p = 0.158$	$F = 1.242_{2,62} p = 0.296$
Dark N <sub>2</sub>	$F = 6.164_{3,55} p = 0.001$ (Winter, spring, summer < late summer)	$F = 1.692_{1,55} p = 0.199$	$F = 0.414_{1,55} p = 0.523$
Light N <sub>2</sub>	$F = 3.108_{3,23} p = 0.046$ (Summer, winter, late summer < spring)	$F = 0.299_{1,23} p = 0.590$	–
Net N <sub>2</sub>	$F = 2.157_{3,19} p = 0.127$	$F = 0.000_{1,19} p = 0.986$	–

structures (West et al., 2006). To determine the influence of mass coral spawning on benthic fluxes, a repeated-measures ANOVA was carried out over the first 7 days of the summer period for each combination of benthic flux (i.e. NH<sub>4</sub><sup>+</sup>, NO<sub>3</sub><sup>-</sup>, O<sub>2</sub>, and N<sub>2</sub>) and treatment (i.e. dark, light and net for all fluxes and also net primary production (NPP), gross primary production (GPP) and GPP/respiration (*p/r*) ratio). A statistical test could only be applied to the first 7 days, as there was no chamber replication for days 8 to 12 (Table 1). Where a significant difference was found among the first 7 days of the summer period, Tukey's HSD post hoc tests were carried out to determine homogenous subsets. To determine differences

between dark and light fluxes, paired *t* tests were carried out. All analysis was carried out using SPSS v20 with  $\alpha = 0.05$ .

### 3 Results

#### 3.1 Benthic fluxes in very coarse and coarse sands, dark versus light and different seasons

None of the fluxes were significantly different between coarse and very coarse sands, except for the NO<sub>3</sub><sup>-</sup> fluxes, which were all higher in the very coarse sands (Table 2) (data of coarse sands not shown). There were season and sediment type interactions for dark O<sub>2</sub> (respiration), gross

**Table 3.** Results from paired *t* test to determine differences in dark and light fluxes.

	NH <sub>4</sub> <sup>+</sup>	NO <sub>3</sub> <sup>-</sup>	DO	N <sub>2</sub>
<i>t</i>	3.605	9.057	32.173	11.961
<i>p</i>	0.001	< 0.001	< 0.001	0.007
difference	Dark < Light	Dark < Light	Dark < Light	Light < Dark

benthic primary production (GPP) and dark NO<sub>3</sub><sup>-</sup> (Table 2). In winter, respiration and GPP were higher in the very coarse sediments than the coarse sediments, and in late summer respiration and GPP were higher in the coarse sediments than the very coarse sediments (Table 2). Dark NO<sub>3</sub><sup>-</sup> fluxes were smaller in the coarse sediment than the very coarse sediments in late summer (Table 2).

There was a significant difference between all dark and light benthic fluxes (Table 3). The sediments consumed O<sub>2</sub> in the dark (respiration) and produced O<sub>2</sub> in the light (NPP) (Fig. 1). NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> fluxes generally showed uptakes in the dark and smaller uptakes or effluxes in the light (Figs. 2 and 3). N<sub>2</sub> effluxes were mostly reduced in the light (Fig. 4).

All benthic fluxes showed a significant difference between seasons, except light NH<sub>4</sub><sup>+</sup>, light NO<sub>3</sub><sup>-</sup> and net N<sub>2</sub> fluxes (Table 2). Dark O<sub>2</sub> uptakes (respiration), GPP, NPP and GPP/dark O<sub>2</sub> (*p/r*) ratios were all higher in late summer and summer than the other seasons (Fig. 1). NH<sub>4</sub><sup>+</sup> and NO<sub>3</sub><sup>-</sup> fluxes all showed greater uptakes in summer than the other seasons (Figs. 2 and 3). Dark N<sub>2</sub> effluxes were highest in late summer, but light N<sub>2</sub> effluxes were highest in spring (Fig. 4).

### 3.2 Daily and episodic variations in benthic fluxes (summer)

Major coral spawning occurred on the night of 7 and 8 December 2010 with *Montipora digitata* and massive corals (e.g., *Platygyra daedalea*) releasing eggs. There was a visible slick of spawning material on the water surface that night, but by the next day little of this material could be seen. There was no large accumulation of spawning material along beaches and in the water column, and only a minor bloom of benthic dinoflagellates (visual observation). Major coral spawning occurred after the benthic chambers were sealed on day 4 in summer; as such, the chamber incubations on days 1 to 4 reflect pre-spawning and days 5 to 12 reflect post-spawning.

The study site experienced a semi-diurnal tide with the average depth of water in the light hours between 8.00 and 16.00 h decreasing from day 1 to 7 and then increasing to day 12. The depth of water at 12.00 h decreased from a maximum on day 1 of 2.36 m to a minimum of 0.28 m on days 6 and 7. Water depth was an important control on PAR measured at the top of the chamber with the highest light levels occurring on days 6 (1685 μE m<sup>-2</sup> s<sup>-1</sup>) and 7 (1717 μE m<sup>-2</sup> s<sup>-1</sup>) when the water depth was shallowest.

*p/r* ratios (*p* < 0.001) and dark NH<sub>4</sub><sup>+</sup> fluxes (*p* = 0.006) showed a significant difference between the pre- and post-spawn periods (Figs. 1 and 2). *p/r* ratios were similar for the first 3 days; then they increased until day 7 and then decreased again to day 12. Dark NH<sub>4</sub><sup>+</sup> uptakes were greater on days 5–7 (post-spawn) than days 1–4 (pre-spawn). The dark NH<sub>4</sub><sup>+</sup> uptakes peaked on day 10 and then decreased to day 12.

Dark O<sub>2</sub> fluxes (benthic respiration) varied significantly over the summer period (*p* < 0.001) with days 2, 3 and 4 having significantly higher uptake rates than days 1, 5, 6 and 7 (Fig. 1). NPP (*p* < 0.001) varied significantly over the summer period, increasing from day 1 to day 6 (Fig. 1). NPP then remained elevated for days 6 to 9 before decreasing again from day 10 to day 12. Dark NO<sub>3</sub><sup>-</sup> uptake rates were significantly different over the summer period (*p* < 0.001) with day 7 having greater uptake rates than the previous 6 days, and day 3 having significantly lower uptake rates than any other day (Fig. 3). There was no significant difference in N<sub>2</sub> fluxes over the first 7-day period (*p* = 0.086), but dark N<sub>2</sub> rates increased greatly on days 11 and 12 (Fig. 4).

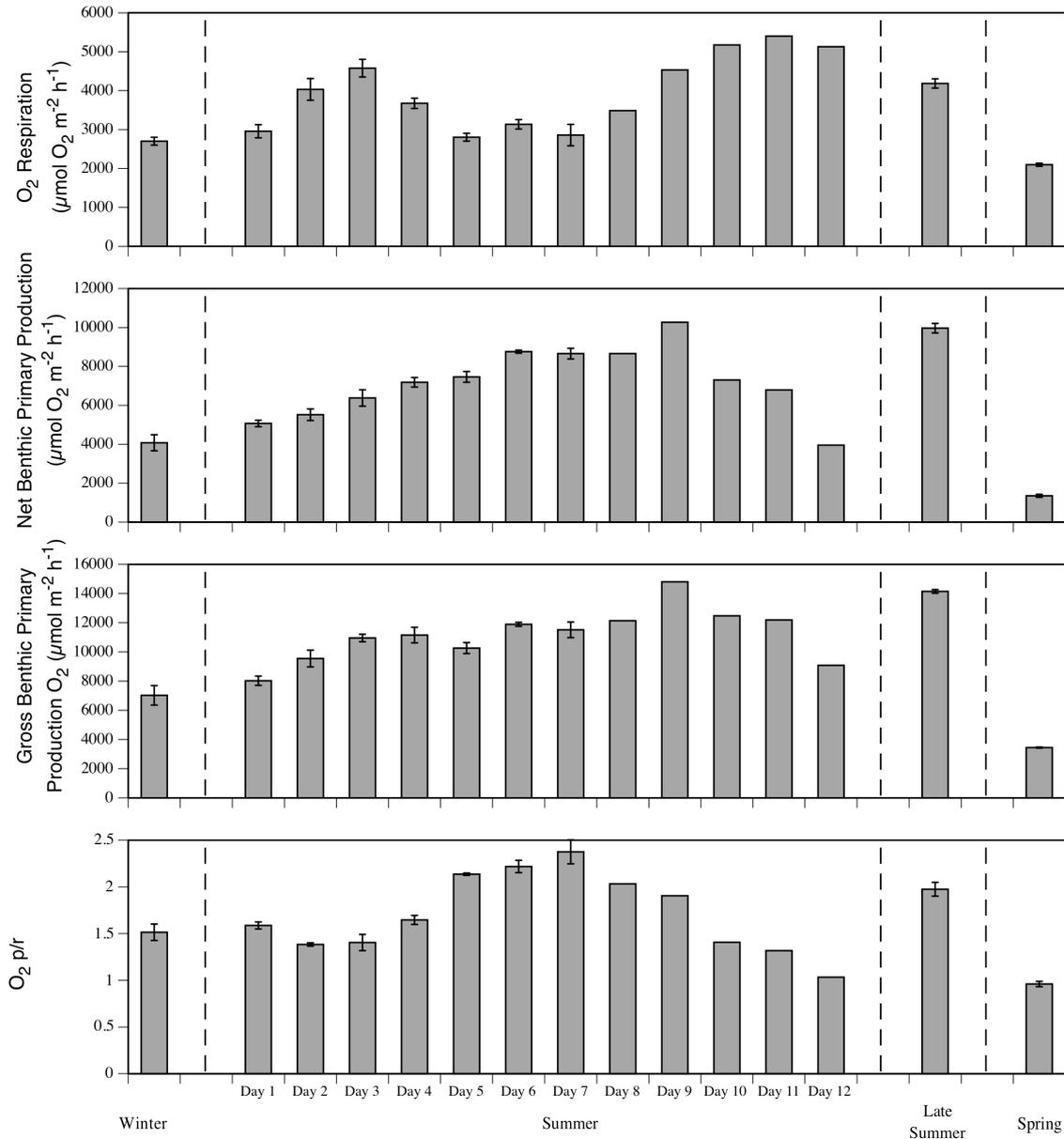
## 4 Discussion

### 4.1 Comparison of denitrification rates in carbonate sands

There have been few measurements of denitrification in carbonate sediments; these have all been in the dark in coral reef sediments, and most of these have been ex situ (Table 4). In sandy sediments only the previous study of Eyre et al. (2008) simulated the effect of advective pore water flow (Table 4). Denitrification rates measured using C<sub>2</sub>H<sub>2</sub> blockage were all much lower than all of the rates of Eyre et al. (2008) and those measured during this study (Table 4). The lower rates measured using C<sub>2</sub>H<sub>2</sub> blockage most likely reflect inhibition of nitrification (Hynes and Knowles, 1978), which would result in rates being underestimated because of the strong nitrification–denitrification coupling (see later discussion). In addition, in the C<sub>2</sub>H<sub>2</sub> blockage slurries there is no advective pore water flow, which appears to enhance coupled nitrification–denitrification in permeable carbonate sediments (Eyre et al., 2008; Santos et al., 2012a). Upper rates of denitrification measured using the direct N<sub>2</sub> flux technique in carbonate muds and sands without advective flow (Alongi et al., 2006, 2008, 2011) approached the pre-spawning rates of Eyre et al. (2008) and the lower rates of this study but were still much lower than the higher rates of Eyre et al. (2008) and this study (Table 4). The dark summer rates were similar to the rates measured 1 to 3 days post-coral-spawning by Eyre et al. (2008) at the same site at the same time of year using exactly the same technique (Table 4), but also lower than the upper rates measured by Eyre et al. (2008) 4 to 5 days post-coral spawning. Williams et al. (1985) estimated that

**Table 4.** Denitrification rates in carbonate sediments.

Rate ( $\mu\text{mol N}_2\text{-Nm}^{-2}\text{h}^{-1}$ )	System	Season	Method	Source
19 (dark)	Sands, Bahamas		Dark, C <sub>2</sub> H <sub>2</sub> , slurry	Seitzinger and D'Elia (1985)
0.12 to 0.45 (dark)	Sands, Hopkinson Reef, GBR		Dark, C <sub>2</sub> H <sub>2</sub> , slurry	Capone et al. (1992)
0.2 to 13.3 (dark)	Sands, Bowl Reef, GBR		Dark, C <sub>2</sub> H <sub>2</sub> , slurry	Capone et al. (1992)
1.7 to 6.5 (dark)	Sands, Ishigaki Island		Dark, C <sub>2</sub> H <sub>2</sub> , slurry	Miyajima et al. (2001)
17 to 37 (dark)	Carbonate muds, Arlington Reef, GBR		Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2006)
7 to 83 (dark)	Carbonate muds, Sudbury Reef, GBR		Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2006)
3 to 67 (dark)	Fine to medium inter-reef channel sands	Winter	Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2008)
61 to 94 (dark)	Fine to medium inter-reef channel sands	Summer	Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2008)
60 to 85 (dark)	Very coarse outer reef channel sands	Spring	Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2011)
19 to 65 (dark)	Very coarse outer reef channel sands	Summer	Dark, Direct N <sub>2</sub> , ex situ chamber, no advective flow	Alongi et al. (2011)
34 to 92 (dark)	Pre-spawning, very coarse permeable sands, Heron Island, GBR	Summer	Dark, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	Eyre et al. (2008)
96 to 152 (dark)	1–3 days post-spawning, very coarse permeable sands, Heron Island, GBR	Summer	Dark, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	Eyre et al. (2008)
400 to 480 (dark)	4–5 days post-spawning, very coarse permeable sands, Heron Island, GBR	Summer	Dark, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	Eyre et al. (2008)
54 (dark) 66 (light) 60 (net)	Very coarse permeable sands, Heron Island, GBR	Winter	Dark, light, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	This study
80–214 (dark) 2–14 (light) 7–70 (net)	Very coarse permeable sands, Heron Island, GBR	Summer	Dark, light, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	This study
234 (dark) 52 (light) 128 (net)	Very coarse permeable sands, Heron Island, GBR	Late Summer	Dark, light, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	This study
82 (dark) 344 (light) 234 (net)	Very coarse sands, Heron Island, GBR	Spring	Dark, light, N <sub>2</sub> : Ar, in situ chamber, advective flow, 40 rpm	This study



**Fig. 1.** Benthic dark O<sub>2</sub> consumption (respiration), light O<sub>2</sub> fluxes (net primary production (NPP), gross primary production (GPP) and GPP/respiration (*p/r*) (mean ± SE where replicated; see Table 1).

denitrification rates of a similar range to those measured in Eyre et al. (2008) and this study (60 to 304 μmol N m<sup>-2</sup> h<sup>-1</sup>) must have been occurring to account for the missing nitrogen flux in coral reef sands at St. Croix.

Because of the strong dependence of denitrification rates on advective flow (Cook et al., 2006; Eyre et al., 2008), and because we do not know the in situ advective pore water flow, the “true” in situ denitrification rate is unknown. The “true” in situ rates of denitrification in carbonate sands would probably vary rapidly due to changes in the interaction of organic matter deposition and drivers of advective pore water flow such as wave and tidal pumping and flow- and

topography-induced advection (see Santos et al., 2012b, for review of the drivers of advective flow in permeable sands). Any measured rate would only represent a chamber-induced transient state. Overall, however, denitrification rates have probably previously been underestimated in carbonate sediments (permeable sands) due to methodological constraints (inhibition of nitrification; no advective pore water flow), and because episodic events that enhance the supply of organic matter to the benthos and stimulate denitrification (e.g. coral spawning, phytoplankton blooms) have only once been previously captured (Eyre et al., 2008). The rates measured in permeable carbonate sands were at the upper end of coupled

**Table 5.** Slope of the relationship between benthic oxygen respiration ( $\mu\text{mol O}_2 \text{ m}^{-2} \text{ h}^{-1}$ ) and dark N<sub>2</sub> efflux ( $\mu\text{mol N}_2\text{-N m}^{-2} \text{ h}^{-1}$ ). Regressions were forced through zero as there would be no N<sub>2</sub> efflux when respiration equals zero and to allow comparisons with previous studies. Outliers were removed from all regressions (see Discussion).

Season	Sediment Type	Slope	$r^2$	$p$	Source
All	Coarse and very coarse permeable carbonate sands	0.035	0.63	< 0.005	This study
Winter	Coarse and very coarse permeable carbonate sands	0.019	0.62	Not Sig.	This study
Summer	Very coarse permeable carbonate sands	0.036	0.42	< 0.05	This study
Late Summer	Coarse and very coarse permeable carbonate sands	0.047	0.43	Not Sig.	This study
Summer	Very coarse permeable carbonate sands	<sup>1</sup> 0.033	0.54	< 0.05	Eyre et al. (2008)
Winter (July)	Fine to medium inter-reef carbonate channel sands	<sup>2</sup> 0.021	0.89	< 0.05	Alongi et al. (2008)
All seasons	Fine to very coarse inter- and outer-reef and carbonate sands	<sup>3</sup> 0.025	0.44	Not Sig.	Alongi et al. (2008, 2011)
All seasons above	All sands above	0.035	0.71	< 0.005	This study; Eyre et al. (2008); Alongi et al. (2008, 2011)
Outliers, all seasons above (see Fig. 6)	Outliers, all sands above (see Fig. 6)	0.089	0.82	< 0.01	This study; Eyre et al. (2008); Alongi et al. (2008, 2011)
Summer	Subtropical muds, range of habitats from subtidal shoals to seagrass	<sup>4</sup> 0.036	<sup>4</sup> 0.90	< 0.01	Eyre et al. (2011)
Range	Subtropical to temperate continental shelf muds	0.116	0.64	< 0.001	Seitzinger and Giblin (1996)
Range	Subtropical to temperate muds and sands	0.086	0.30	< 0.01	Fennel et al. (2009)
Range	Temperate muds, range of habitats from subtidal shoals to seagrass	0.036 to 0.107 0.063 (average)	0.67 to 0.94	< 0.001	Piehler and Smyth (2011)

<sup>1</sup> Data from Fig. 4 in Glud et al. (2008) and Fig. 3 in Eyre et al. (2008); <sup>2</sup> Data from sites KR, PR, MR, OB and IR in Tables 4 and 8 in Alongi et al. (2008); <sup>3</sup> Alongi et al. (2008) and data from site PRC4 and OS1B in Table 3 and Fig. 8 in Alongi et al. (2011); <sup>4</sup> Data from Fig. 5 in Eyre et al. (2011),  $r^2$  is slightly less than in Eyre et al. (2011) due to forcing through zero.

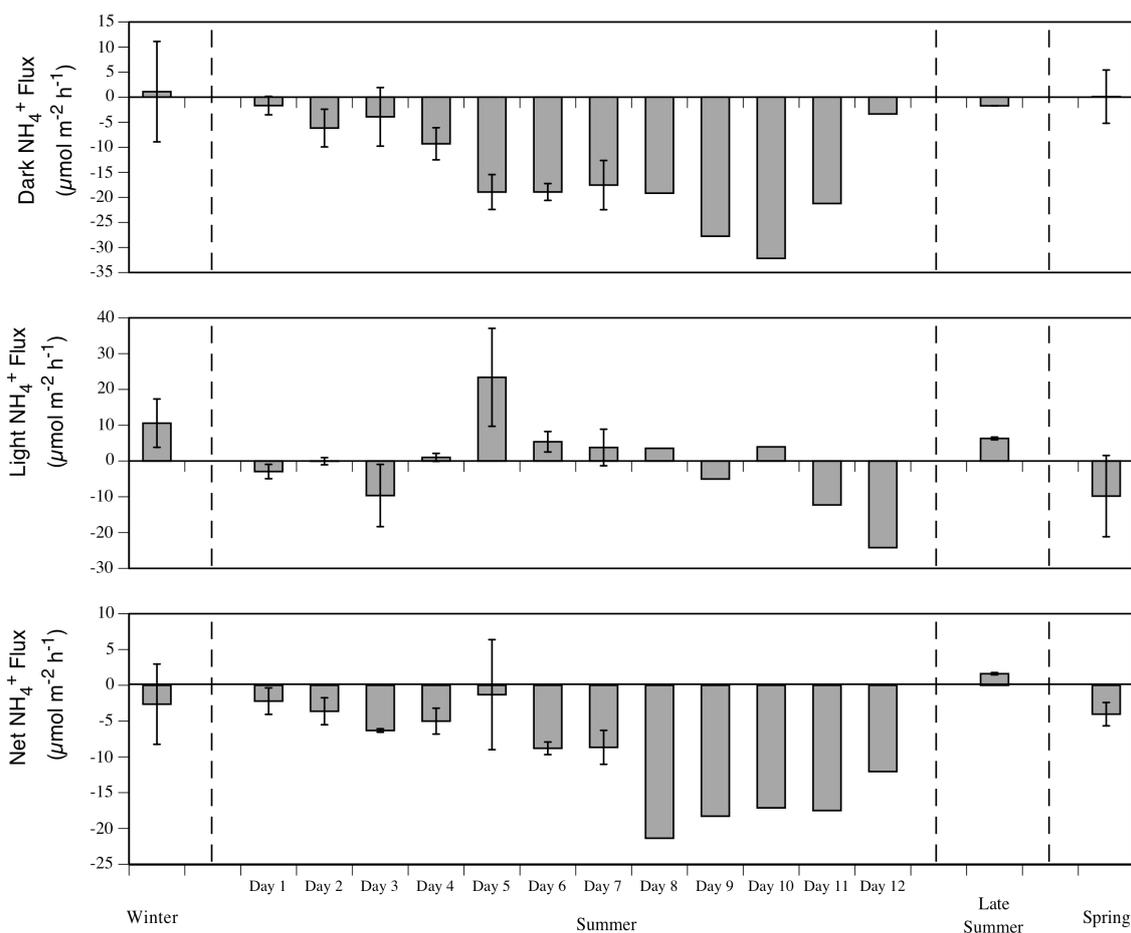
nitrification–denitrification rates measured in non-carbonate muddy sediments (Fennel et al., 2009), but similar to the range measured in permeable quartz sands (Gao et al., 2012).

#### 4.2 Effect of mass coral spawning on benthic fluxes

There was no distinct response of the benthic system to mass coral spawning (e.g. enhanced respiration, GPP, NPP, and NH<sub>4</sub><sup>+</sup> effluxes) as has been previously observed (e.g. Wild et al., 2004; Glud et al., 2008; Eyre et al., 2008). This is consistent with the lack of a large accumulation of spawning

material along beaches and in the water column, and only a minor bloom of benthic dinoflagellates. In contrast, previous observations of mass coral-spawning events at Heron have seen large accumulations of coral-spawning material along the beaches and large blooms of benthic dinoflagellates (see Fig. 1C and D in Glud et al., 2008).

Even the significant effects of dark NH<sub>4</sub><sup>+</sup> uptakes and  $p/r$  ratios between pre- and post-spawning are more likely driven by changes in water depth and PAR over the 12-day summer study period. For example, NPP is highly correlated with average hourly PAR received at the top of the chamber ( $r^2 =$



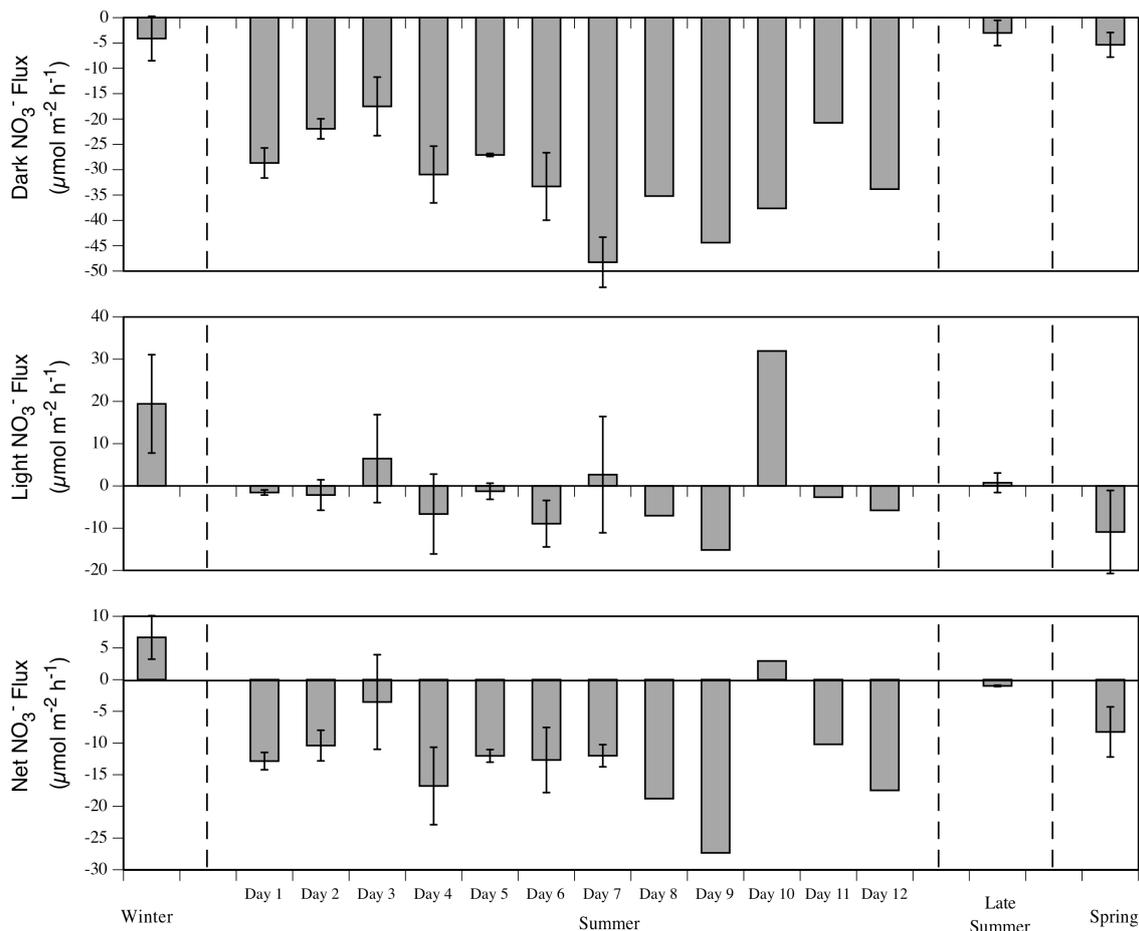
**Fig. 2.** Benthic dark, light, and net ammonium (NH<sub>4</sub><sup>+</sup>) fluxes (mean ± SE where replicated; see Table 1).

0.79;  $p \leq 0.001$ ;  $n = 26$ ) and water depth at 13.00 h ( $r^2 = 0.82$ ;  $p \leq 0.001$ ;  $n = 26$ ), and as expected PAR is highly dependent on water depth ( $r^2 = 0.81$ ;  $p \leq 0.01$ ;  $n = 26$ ). Dark NH<sub>4</sub><sup>+</sup> uptakes and NPP are positively correlated ( $r^2 = 0.55$ ;  $p \leq 0.01$ ;  $n = 26$ ), reflecting the dark uptake of NH<sub>4</sub><sup>+</sup> by benthic microalgae. A clear pattern of NH<sub>4</sub><sup>+</sup> uptake in the light is not seen because water column NH<sub>4</sub><sup>+</sup> concentrations are so low during the light (Eyre et al., 2008) that the benthic fluxes are not easily measured (see variability in Fig. 2). Larger NH<sub>4</sub><sup>+</sup> uptakes in summer during coral mass spawning, compared to winter and spring, probably reflect higher rates of NPP, although this is not consistent with late summer when NPP is high and NH<sub>4</sub><sup>+</sup> uptake is low. It is unknown if the lack of coral-spawning material is due to less material being produced by the corals or a physical effect such as wind direction that accumulates spawning material in the lagoon. Inter-annual variations in the response of coral reefs, and in particular the benthic system, to mass coral spawning is an area for further research.

### 4.3 Organic matter control of denitrification

Dark N<sub>2</sub> fluxes were strongly controlled by benthic respiration (Fig. 5; Table 5), most likely reflecting an increased supply of NH<sub>4</sub><sup>+</sup> from ammonification for coupled nitrification–denitrification and the availability of electron donors for denitrification. NO<sub>3</sub><sup>−</sup> uptakes were not significantly correlated with N<sub>2</sub> fluxes due to the very low water column NO<sub>3</sub><sup>−</sup> concentrations (< 1 μmol L<sup>−1</sup>; Eyre et al., 2008), providing further support for a dominance of coupled nitrification–denitrification in these sediments. Benthic respiration and NPP were not correlated ( $r^2 = 0.08$ ), indicating that other sources of organic matter, probably in addition to benthic microalgae, must have been driving benthic respiration. Possible sources include coral mucus, turf algae, coral-spawning material and phytoplankton detritus, which have previously been suggested as drivers of benthic respiration and denitrification at this site (Eyre et al., 2008).

The relationship between benthic respiration and dark N<sub>2</sub> efflux is significant if data from all seasons and sites (coarse and very coarse sands) are included (Fig. 5; Table 5). However, there were seasonal differences with a steeper slope in



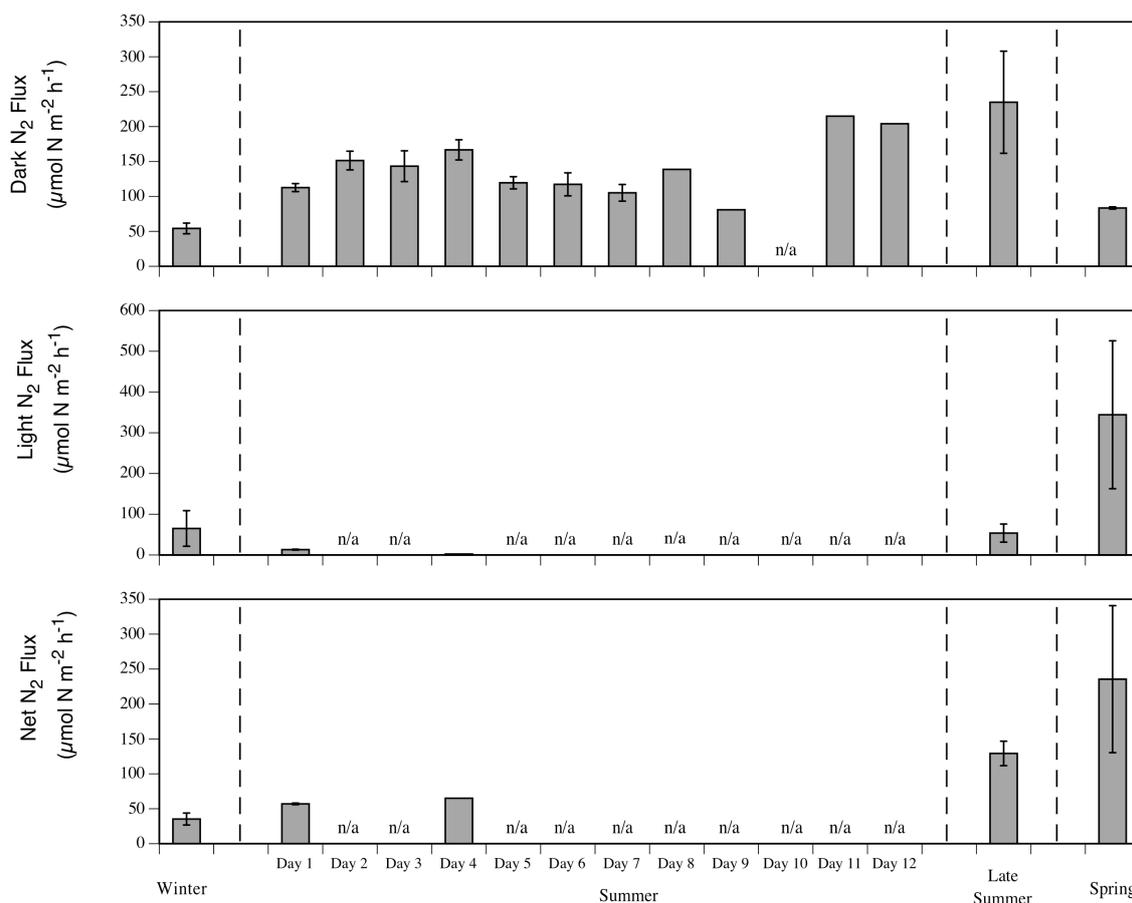
**Fig. 3.** Benthic dark, light, and net nitrate ( $\text{NO}_3^-$ ) fluxes (mean  $\pm$  SE where replicated; see Table 1).

summer than winter (Table 5). The steeper summer slope probably reflects a more efficient coupling between respiration and nitrification–denitrification at higher temperatures or a difference in the source of organic matter driving benthic respiration. Seasonal differences in the relationship between benthic respiration and denitrification were also seen in a shallow temperate coastal system, but steeper slopes occurred in winter and summer compared to spring and autumn (Piehler and Smyth, 2011; Table 5), indicating that temperature was not an important driver of the differences. Despite seasonal differences in the relationship between benthic respiration and dark N<sub>2</sub> efflux, the relationship for a given season appears consistent inter-annually as shown by similar summer slopes in this study and a study at the same site in 2005 (Eyre et al., 2008; Table 5).

Including all the data from tropical carbonate sands improves the strength and significance of the relationship between benthic respiration and dark N<sub>2</sub> efflux (Fig. 6; Table 5). Despite improving the relationship, the inter-reef carbonate sands generally had lower rates of benthic respiration and associated N<sub>2</sub> efflux, which may be due to either a lack of

advective flow during the measurements or the greater depth of the sample sites and therefore less organic matter deposition, or a combination thereof (Alongi et al., 2008). The slope of the combined data for tropical carbonate sands is similar to that found across some subtropical sub-tidal and inter-tidal muddy habitats (Table 5) and temperate sub-tidal muddy habitats (lowest temperate habitat slope; Table 5). This demonstrates that benthic respiration and associated coupled nitrification–denitrification in carbonate sands can be just as efficient as in some muddy sediments. However, the slope is lower than that found in most muddy temperate and subtropical coastal and continental shelf sediments (Table 5).

The lower slope of the relationship between benthic respiration and dark N<sub>2</sub> efflux in tropical carbonate sediments may be due to one or a combination of the following: competition with benthic microalgae for nitrogen, less-efficient coupled respiration–nitrification–denitrification in sands, or the composition of organic matter driving respiration and associated assimilation of nitrogen by heterotrophic bacteria, including enhanced N-fixation. Support for a reduction in N<sub>2</sub> efflux due to competition from benthic microalgae is given by the



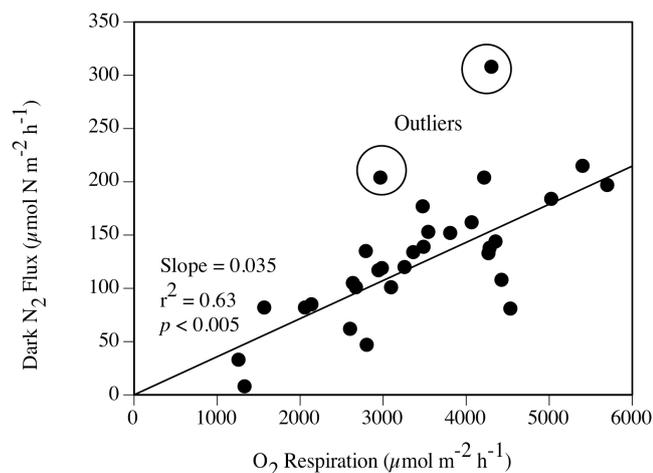
**Fig. 4.** Benthic dark, light, and net dinitrogen gas (N<sub>2</sub>) fluxes (mean ± SE where replicated; see Table 1). n/a = no data available due to O<sub>2</sub> saturation of the chamber water (see Methods).

negative correlation between dark N<sub>2</sub> efflux and dark NH<sub>4</sub><sup>+</sup> ( $r^2 = 0.34$ ,  $p = 0.05$ ,  $n = 29$ ) and NO<sub>3</sub><sup>-</sup> ( $r^2 = 0.24$ , not significant,  $n = 29$ ) uptakes, and dark N<sub>2</sub> efflux and benthic productivity/respiration ( $p/r$ ) ( $r^2 = 0.56$ ,  $p < 0.01$ ,  $n = 29$ ).

Phytoplankton production is typically low in oligotrophic coral reef environments (Furnas et al., 2005), highlighting the possible role of other sources of organic matter, such as coral mucus, turf algae, macrophytes and coral-spawning material, in driving benthic respiration. These sources of organic matter have higher carbon:nitrogen (C:N) ratios than phytodetritus, resulting in less nitrogen being released as N<sub>2</sub> for a given amount of respiration due to competition for nitrogen by heterotrophic bacteria (Oakes et al., 2011). N-limitation of the microbial decomposition of high C:N organic material results in the uptake and accumulation of nitrogen by bacteria (Tupas and Koike, 1991; van Duyf et al., 1993; Lomstein et al., 1998). Several types of bacteria can assimilate NH<sub>4</sub><sup>+</sup>, including sulphate reducers and fermentative bacteria (Koike and Sumi, 1989). Additionally, sulphate reducers can fix nitrogen (Nielsen et al., 2001). N<sub>2</sub> effluxes are a measure of denitrification minus N-fixation; as such, increased N-fixation will result in a reduced N<sub>2</sub> efflux. Further, cou-

pled nitrification–denitrification may be suppressed by H<sub>2</sub>S produced during sulphate reduction (Joye and Hollibaugh, 1995; Fulweiler et al., 2013). Consistent with higher rates of heterotrophic N-fixation during decomposition of higher C:N organic material is the post-spawning reduction in dark N-fixation rates at this study site when low C:N phytodetritus was deposited (Eyre et al., 2008).

Further, C:N ratios of sediment trap material at the Heron Island reef lagoon study site ranged from 10.5 to 17.2 (Eyre et al., 2008) and sediment C:N ratios at the inter-reef channel sites ranged from 8.1 to 16.8 (Alongi et al., 2008), which is higher than typical fresh phytodetritus (Redfield 6.6). Remineralisation ratios (O<sub>2</sub> respiration/(N<sub>2</sub>-N+NH<sub>4</sub><sup>+</sup>+NO<sub>3</sub><sup>-</sup>)) of the data in Fig. 6(excluding outliers) ranged from 15 to 65 and averaged 37, demonstrating that high C:N organic matter was being respired. The remineralisation ratios were always higher than the organic matter substrate, which is consistent with assimilation of nitrogen by heterotrophic bacteria, including N-fixation. High C:N organic matter may also favour anammox over denitrification due to denitrification not proceeding all the way to N<sub>2</sub> and associated leakage of NO<sub>2</sub> that fuels anammox

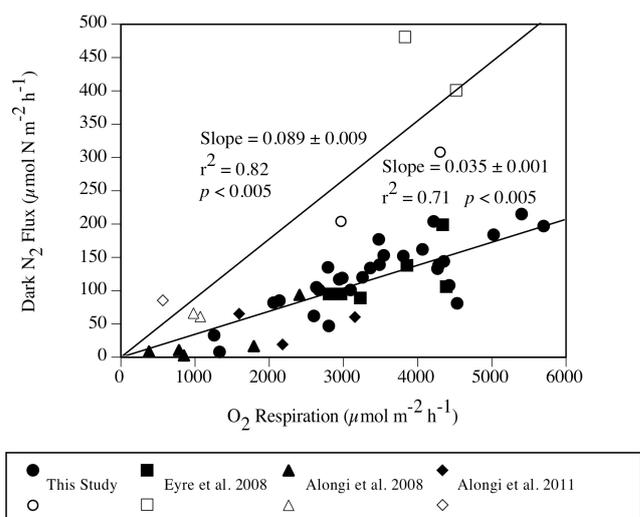


**Fig. 5.** Dark N<sub>2</sub> effluxes versus benthic respiration for all seasons in very coarse and coarse sands (individual chambers).

(Trimmer and Nicholls, 2009; Erler et al., 2013). Anammox was found at some of the inter-reef sites (Erler et al., 2013) but not in the Heron Island reef lagoon (Eyre et al., 2008). However, if anammox was quantitatively important then the end result would be more N<sub>2</sub> production for a given benthic respiration, not less (i.e. lower slope).

Further support for the low slope of the relationship between benthic respiration and dark N<sub>2</sub> efflux being driven by high C:N organic material is given by the outliers in each of the three data sets used to develop the relationship in tropical carbonate sands (Fig. 6). The outliers fell outside the upper 95 % confidence limit for the regressions of the 3 data sets (Alongi et al., 2008, 2011; Eyre et al., 2008). The slope of the outliers is significantly different from the lower slope (Student's *t* test;  $p < 0.05$ ) and is similar to the slopes for most of the non-tropical muds and sands (Seitzinger and Giblin, 1996; Fennel et al., 2009; Table 5). The outliers from Eyre et al. (2008) were due to the deposition of phytodetritus, suggesting the higher slope of the outliers' data (Fig. 6) and the non-tropical sands and muds (Table 5) are driven by the deposition of nitrogen rich phytodetritus. The remineralisation ratios ( $O_2$  respiration/ ( $N_2-N+NH_4^++NO_3^-$ )) of the outliers ranged from 5.0 to 14.2 and averaged 7.0, which is very close to that expected for phytodetritus, and no heterotrophic assimilation of nitrogen due to low C:N. In addition, flow-through reactor experiments using permeable carbonate sands from the same site as this study, and seawater with phytoplankton as the organic matter source, had a benthic respiration versus dark N<sub>2</sub> efflux slope of 0.114 (Santos et al., 2012a), which is also similar to the outliers' slope (Table 5).

Modelling and flume studies have suggested that permeable quartz sands have a low respiration–nitrification–denitrification efficiency ( $(N_2-N/Dark\ O_2\ Flux/6.6) \times 100\%$ ) with a maximum loss of about 15 % or



**Fig. 6.** Dark N<sub>2</sub> effluxes versus benthic respiration for carbonate sands from this study (individual chambers), Eyre et al. (2008) (individual chambers) and Alongi et al. (2008, 2011). See Table 4 for sites used from Alongi et al. (2008, 2011). Open symbols are outliers that fall above the upper 95 % confidence interval of the regressions of each of the 3 data sets above (see Discussion).

less as N<sub>2</sub> of the remineralised nitrogen for Redfield organic matter (Cardenas et al., 2008; Kessler et al., 2012). Using the benthic respiration versus dark N<sub>2</sub> slope of 0.035 for tropical carbonate sands gives losses of 23, 42 and 71 % as N<sub>2</sub> of the remineralised nitrogen for organic matter C:N ratios of Redfield (6.6), coral mucus (12.0; Wild et al., 2004) and coral-spawning material (20.3; Eyre et al., 2008) ( $(Dark\ O_2\ Flux \times 0.035)/Dark\ O_2\ Flux/C:N \times 100\%$ ), respectively, which are all higher than the modelling and flume studies. Similarly, using the higher slope (0.088) and Redfield organic matters gives a loss of 58 % as N<sub>2</sub>. At a carbon mineralisation rate of  $1133\ \mu mol\ m^{-2}\ h^{-1}$  the permeable quartz sands model estimated a maximum denitrification rate of  $30\ \mu mol\ N\ m^{-2}\ h^{-1}$  (Cardenas et al., 2008), which is also lower than the denitrification rate of  $40\ \mu mol\ N\ m^{-2}\ h^{-1}$  estimated using the 0.035 slope and  $100\ \mu mol\ N\ m^{-2}\ h^{-1}$  using the 0.088 slope. The difference between the modelled and measured respiration–nitrification–denitrification efficiencies and denitrification rates may be due to the unique interaction of advection and biogeochemistry in permeable carbonate sands (see Santos et al., 2012a) not captured in the model, or to the model simply under-estimating denitrification rates in permeable sediments (see high rates in Gao et al., 2012).

Permeable carbonate sands appear to be just as efficient as muds in coupling between respiration and nitrification–denitrification with rates of denitrification controlled by the quantity and quality of organic matter driving respiration (Fig. 6). Most importantly, a high denitrification efficiency ( $\%N_2-N/(N_2-N+NH_4^++NO_3^-)$  efflux) is maintained even

at high respiration rates ( $> 5000 \mu\text{mol m}^{-2} \text{h}^{-1}$ ). In contrast, the denitrification efficiency of muds decreases rapidly above respiration rates of around  $1500 \mu\text{mol m}^{-2} \text{h}^{-1}$  (Eyre and Ferguson, 2009). Recent flow-through reactor (FTR) experiments with permeable carbonate sediments give some insight into the possible drivers of this high efficiency (Santos et al. 2012a). Three-dimensional microenvironments within the porous carbonate grains may enhance the area, compared to quartz grains, where microbial communities grow and biogeochemical reactions can occur. The increase in coupled nitrification–denitrification as advection increases (Eyre et al., 2008) is due to partial flushing of the sands with oxygenated pore water and the development of sharp oxic-anoxic gradients between the inner and outer parts of the carbonate grains. The FTRs showed a decrease in coupled nitrification–denitrification at high advective flows, which was not seen in the benthic chambers at the advective flows used (Eyre et al., 2008), due to complete flushing of the sands with oxygenated porewater that prevented the development of oxygen-depleted microenvironments within grains (Santos et al., 2012a).

#### 4.4 Missing nitrogen on the reef

Although easily explained by the high respiration rates in the shallow productive environment, denitrification rates in the coral reef carbonate sands were very high for such a nitrogen-poor environment. Carbonate sands cover about 85 % of the Heron Island lagoon (Glud et al., 2008). With such a large loss of nitrogen via denitrification, combined with net autotrophic production in the sediments ( $p/r > 1$ ), the question has to be asked, “from where does the nitrogen that supports denitrification and benthic production come”? Possible sources include cross-reef advection of large volumes of water (Atkinson, 1992), N-fixation by coral symbionts (Lesser et al., 2007) and particles from the ocean catchment (Wyatt et al., 2010). Another process that could account for the large nitrogen input required to balance the large nitrogen losses via benthic denitrification is unmeasured light N-fixation in the sediments.

The few measurements of light N<sub>2</sub> fluxes are generally much lower than dark N<sub>2</sub> effluxes probably due to N-fixation by cyanobacteria in the microphytobenthos. Alternatively, competition for nitrogen with benthic microalgae in the microphytobenthos that are typically nitrogen-limited tropical oligotrophic coral reef environments (Dizon and Yap, 1999; Clavier et al., 2005) may have reduced the light N<sub>2</sub> fluxes. The resultant net N<sub>2</sub> fluxes would result in far less nitrogen loss from the system than would be estimated using dark N<sub>2</sub> effluxes. It also needs to be noted that these rates are only for the first few hours of the day. However, if these rates were measured over the full light cycle we suspect that the sediments would be net N<sub>2</sub> sinks with N-fixation in the light exceeding denitrification in the dark, but this cannot be determined using the N<sub>2</sub>/Ar method. Unfortunately, bubbles are

produced during the light in the highly productive sediments, which reduces the N<sub>2</sub>/Ar ratio (see Discussion in Eyre et al., 2002), giving a pseudo-rate of N-fixation. As such, most of the light N<sub>2</sub>/Ar data in this study, and all of the light N<sub>2</sub>/Ar data in the previous study at this site (Eyre et al., 2008), had to be excluded. Measured N-fixation rates in coral reef sediments are low ( $< 26.0 \mu\text{mol N}_2\text{-N m}^{-1} \text{h}^{-1}$  reviewed in Capone et al., 1992), including at this site ( $< 37.4 \mu\text{mol N}_2\text{-N m}^{-1} \text{h}^{-1}$ ; Eyre et al., 2008), but these have all been measured using slurries in the dark and exclude advection and diel cycles, which may be important drivers of N-fixation; earlier measurements of denitrification that excluded advection and diel cycles were also much lower (Table 4). Further work is required to better measure N-fixation in permeable coral reef carbonate sands over diel cycles with advection.

#### 4.5 Global nitrogen budgets

Continental shelves account for a third to half of global denitrification (Middelburg et al., 1996; Seitzinger et al., 2006). Seventy percent of continental shelves are covered by permeable sediments (Emery, 1968), and around 40 % of the shelves are covered by carbonate sediments (Milliman and Droxler, 1996). There has been some debate over the role of these permeable sediments in global rates of denitrification on the continental shelf. Based on modelling of permeable quartz sands, Cardenas et al. (2008) argue that global rates of continental shelf denitrification are over-estimated by a factor of 2 to 7. In contrast, Gao et al. (2012) suggest that global rates of continental shelf denitrification may be underestimated due to high rates of denitrification measured in permeable quartz sands. The findings from this study suggest that global rates of continental shelf denitrification may be either over-estimated or about right, but not under-estimated (see following discussion).

A recent estimate of denitrification in shelf sediments of  $250 \text{Tg N yr}^{-1}$  (Seitzinger et al., 2006) was based on a slope of 0.116 for benthic respiration and dark N<sub>2</sub> (Table 5; Seitzinger and Giblin, 1996) and is within the range of previous estimates ( $214\text{--}300 \text{Tg N yr}^{-1}$ ; reviewed in Seitzinger et al., 2006). If the lower slope (0.035) for benthic respiration and dark N<sub>2</sub> (Fig. 6; Table 5) is due to less-efficient coupled respiration and nitrification–denitrification in permeable carbonate sediments, it would result in a reduction in global shelf denitrification rates (from Seitzinger et al., 2006 estimates) of around 20 %; if the lower slope applied to all permeable sediments, then it would result in a reduction of around 50 %. The latter decrease is consistent with the smallest of Cardenas et al. (2008) estimates of reduced shelf denitrification (i.e. 2-times reduction). However, if the 0.035 slope is due to high C:N organic matter associated with the coral reef environments, and the higher outliers' slope of 0.089 is applicable to most continental shelves by permeable carbonate sediments that are dominated by phytoplankton production, then global shelf denitrification

rates would only be reduced about 10 %. If the slope of 0.089 is applicable to all permeable sediments on the continental shelf, then global shelf denitrification rates would be reduced about 17 % to 207 Tg N yr<sup>-1</sup>, which is still close to the range of previous estimates.

All estimates of global continental shelf denitrification are based on denitrification rates measured in the dark (Seitzinger et al., 2006). Although Lauren and Seitzinger (2002) measured denitrification in continental shelf sediments over a diel cycle, too few samples ( $n = 3$ ) were taken to see an effect in the light. However, N<sub>2</sub> effluxes might be reduced in the light due to competition with benthic microalgae and higher rates of N-fixation than in the dark (Fig. 4). Because light reaches 33 % of the global shelf area (Gattuso et al., 2006), estimates of global continental shelf denitrification based on dark rates of denitrification may be over-estimated. Future work should be directed at better characterising the relationship between benthic respiration and dark and light (net) N<sub>2</sub> efflux for permeable carbonate and quartz sands. In particular, the interaction between diel cycles in benthic metabolism and denitrification, N-fixation, and net N<sub>2</sub> effluxes and advective porewater flow should be investigated.

**Acknowledgements.** This project was supported by Australian Research Council Discovery grants awarded to BDE (DP0878683 and LP100200732). We thank Dirk Erler for assistance in the field, Iain Alexander for assistance with laboratory work and the staff of the Heron Island Research Station for invaluable support. Two anonymous referees, Tom Jilbert and the Associate Editor provided comments that improved the manuscript.

Edited by: C. Woulds

## References

- Alongi, D. M., Pfitzner, J., and Trott, L. A.: Deposition and cycling of carbon and nitrogen in carbonate mud of the lagoons of Arlington and Sudbury Reefs, Great Barrier Reef, Coral Reefs, 25, 123–143, 2006.
- Alongi, D. M., Trott, L. A., and Pfitzner, J.: Biogeochemistry of inter-reef sediments on the northern and central Great Barrier Reef, Coral Reefs, 27, 407–420, 2008
- Alongi, D. M., Trott, L. A., and Mohl, M.: Strong tidal currents and labile organic matter stimulate benthic decomposition and carbonate fluxes on the southern Great Barrier Reef Shelf, Cont. Shelf Res., 31, 1384–1395, 2011.
- Atkinson, M. J.: Productivity of the Enewetak flats predicted from mass transfer relationships, Cont. Shelf Res., 12, 799–807, 1992.
- Capone, D. G., Dunham, S. E., Horrigan, S. G., and Duguay, L. E.: Microbial nitrogen transformations in unconsolidated coral reef sediments, Mar. Ecol. Prog. Ser., 80, 75–88, 1992.
- Cardenas, M. B., Cook, P. L. M., Jiang, H., and Traykovski, P.: Constraining denitrification in permeable wave-influenced marine sediment using linked hydrodynamic and biogeochemical modeling. Earth Plan. Sci. Lett., 275, 127–137, 2008.
- Clavier, J., Boucher, G., Chauvaud, L., Fichez, R., and Chifflet, S.: Benthic response to ammonium pulses in a tropical lagoon: implications for coastal environmental processes, J. Exp. Mar. Biol. Ecol. 316, 231–241, 2005.
- Cook, P., Wenzhofer, F., Rysgaard, S., Oleksiy, S., Galaktionov, O., Meysman, F. J. R., Eyre, B. D., Cornwell, J., Huettel, M., and Glud, R. N.: Quantification of denitrification in permeable sediments: Insights from a 2 dimensional simulation analysis and experimental data, Limnol. Oceanogr. Methods, 4, 294–307, 2006.
- Cyronak, T., Santos, I. R., McMahon, A., and Eyre, B. D.: Carbon cycling hysteresis in permeable carbonate sands over a diel cycle, Limnol. Oceanogr., 58, 131–143, 2013.
- Dizon, R. M. and Yap, H. T.: Short-term responses of coral reef microphytobenthic communities to inorganic nutrient loading. Limnol. Oceanogr., 44, 1259–1267, 1999.
- Emery, K. O.: Relict sediments on continental shelves of the world. Am. Assoc. Petrol. Geol. Bull., 52, 445–464, 1968.
- Erler, D. V., Trott, L. A., Alongi, D. M., and Eyre, B. D.: Denitrification, anammox and nitrate reduction in the sediments of the Southern Great Barrier Reef Lagoon. Mar. Ecol. Prog. Ser., 478, 57–70, 2013.
- Eyre, B. D. and Ferguson, A. J. P.: Benthic metabolism and nitrogen cycling in a sub tropical east Australian estuary (Brunswick): Temporal variability and controlling factors, Limnol. Oceanogr., 50, 81–96, 2005.
- Eyre, B. D. and Ferguson, A. J. P.: Denitrification efficiency for defining critical loads of carbon in shallow coastal ecosystems, Hydrobiologia, 629, 137–146, 2009.
- Eyre, B. D., Rysgaard, S., Dalsgaard, T., and Christensen, P. B.: Comparison of isotope pairing and N<sub>2</sub>/Ar methods for measuring sediment denitrification rates – assumptions, modifications and implications, Estuaries, 25, 1077–1087, 2002.
- Eyre, B. D., Glud, R. N., and Pattern, N.: Coral mass spawning – a natural large-scale nutrient enrichment experiment, Limnol. Oceanogr., 53, 997–1013, 2008.
- Fennel, K., Brady, D., DiToro, D., Fulweiler, R. W., Gardner, W. S., Giblin, A., McCarthy, M. J., Rao, A., Seitzinger, S., Thouvenot-Korppoo, M., and Tobias, C.: Modeling denitrification in aquatic sediments, Biogeochemistry, 93, 159–178, 2009.
- Fulweiler, R. W., Brown, S. M., Nixon, S. W., and Jenkins, B. D.: Evidence and a conceptual model in heterotrophic marine sediments, Mar. Ecol. Prog. Ser., doi:10.3354/meps10240, 2013.
- Furnas, M. J., Mitchel, A., Skuza, M., and Brodie, J.: In the other 90 %: phytoplankton responses to enhanced nutrient availability in the Great Barrier Reef Lagoon, Mar. Pollut. Bull., 51, 253–265, 2005.
- Gao, H., Matyka, M., Liu, B., Khalili, A., Kostka, J. E., Collins, G., Jansen, S., Holtappels, M., Jensen, M. M., Badewien, T. H., Beck, M., Grunwald, M., de Beer, D., Lavik G., and Kuypers, M. M.: Intensive and extensive nitrogen loss from intertidal permeable sediments of the Wadden Sea, Limnol. Oceanogr., 57, 185–198, 2012.
- Gattuso, J.-P., Gentili, B., Duarte, C. M., Kleypas, J. A., Middelburg, J. J., and Antoine, D.: Light availability in the coastal ocean: impact on the distribution of benthic photosynthetic organisms and their contribution to primary production, Biogeochemistry, 3, 489–513, doi:10.5194/bg-3-489-2006, 2006.
- Glud, R. N., Eyre, B. D., and Pattern, N.: Biogeochemical responses to coral mass-spawning on the Great Barrier Reef: Effects on res-

- piration and primary production, *Limnol. Oceanogr.*, 53, 1014–1024, 2008.
- Gihring, T. M., Canion, A., Riggs, A., Huettel, M., and Kostka, J. E.: Denitrification in shallow, sublittoral Gulf of Mexico permeable sediments, *Limnol. Oceanogr.*, 55, 43–54, 2010.
- Huettel, M. and Gust, G.: Solute release mechanisms from confined sediment cores in stirred benthic chambers and flume flows, *Mar. Ecol. Prog. Ser.*, 82, 187–195, 1992.
- Hynes, R. K. and R. Knowles.: Inhibition by acetylene of ammonia oxidation in *Nitrosomonas europaea*, *FEMS Microbiol. Lett.*, 4, 319–321, 1978.
- Joye, S. B. and Hollibaugh, J. T.: Influence of sulphide inhibition of nitrification on nitrogen regeneration in sediments, *Science*, 270, 623–625, 1995.
- Kessler, A. J., Glud, R. N., Cardenas, M. B., Larsen, M., Bourke, M. F., and Cook, P. L. M.: Quantifying denitrification in rippled permeable sands through combined flume experiments and modeling, *Limnol. Oceanogr.*, 57, 1217–1232, 2012.
- Koike, I. and Sumi, T.: Nitrogen cycling in coastal sediments with special reference to ammonium metabolism, in: *Recent Advances in Microbial Ecology*, edited by: Maruyama, R., Morita, Y. and Uchida, A., Japan Scientific Societies Press, Tokyo, 365–369, 1989.
- Lauren, A. E. and Seitzinger, S. P.: The role of denitrification in nitrogen removal and carbon mineralisation in Mid-Atlantic Bight sediments, *Cont. Shelf Res.*, 22, 1397–1416, 2002.
- Lesser, M.P., Falcón, L. I., Rodríguez-Román, A., Enriquez, S., Hoegh-Guldberg, O., and Iglesias-Prieto, R.: Nitrogen fixation by symbiotic cyanobacteria provides a source of nitrogen for the scleractinian coral *Montastraea cavernosa*, *Mar. Ecol. Prog. Ser.*, 346, 143–152, 2007.
- Lomstein, B. A., Jensen, A., Hansen, J., Andreassen, J. B., Hansen, L. S., Berntsen, J., and Kunzendorf, H.: Budgets of sediment nitrogen and carbon cycling in the shallow water of Knebel Vig, Denmark, *Aquat. Microb. Ecol.*, 14, 69–80, 1998.
- Middelburg, J. J., Soetaert, K., Herman, P. M. J., and Heip, C. H. R.: Denitrification in marine sediments: A model study, *Global Biogeochem. Cy.*, 10, 661–673, 1996.
- Milliman, J. D. and Droxler, A. W.: Neritic and pelagic carbonate sedimentation in the marine environment: ignorance is not bliss, *Geol. Rundsch.*, 85, 496–504, 1996.
- Miyajima, T., Masahiro, S., Umezawa, Y., and Koike, I.: Microbial nitrogen transformation in carbonate sediments of a coral-reef lagoon and associated seagrass beds, *Mar. Ecol. Prog. Ser.*, 217, 273–286, 2001.
- Nielsen, L. B., Finster, K., Welsh, D. T., Donnelly, A., Herbert, R. A., de Wit, R., and Lomstein, B. A.: Sulfate reduction and nitrogen fixation rates associated with roots, rhizomes and sediments from *Zostera noltii* and *Spartina maritima* meadows, *Environ. Microbiol.*, 3, 63–71, 2001.
- Nowicki, B. L.: The effect of temperature, oxygen, salinity and nutrient enrichment on estuarine denitrification rates measured with a modified nitrogen gas flux technique, *Estuar. Coast. Shelf Sci.*, 38, 137–156, 1994.
- Oakes, J. M., Eyre, B. D., and Ross, D. J.: Short-term enhancement, and long-term suppression, of denitrification in estuarine sediments receiving primary- and secondary-treated paper and pulp mill discharge, *Environ. Sci. Technol.*, 45, 3400–3406, 2011.
- Piehl, M. F. and Smyth, A.: Habitat-specific distinctions in estuarine denitrification affect both ecosystem function and services, *Ecosphere*, 2, 1–16, 2011.
- Rao, A. M. F., McCarthy, M. J., Gardner, W. S., and Jahnke, R. A.: Respiration and denitrification in permeable continental shelf deposits on the South Atlantic Bight: Rates of carbon and nitrogen cycling from sediment column experiments, *Cont. Shelf Res.*, 27, 1801–1819, 2007.
- Santos, I. R., Tait, D., Erler, D., and Eyre, B. D.: Breathing of a coral cay: tracing tidally-driven seawater recirculation in permeable coral reef sediments, *J. Geophys. Res.*, 115, C12010, doi:10.1029/2010JC006510, 2010.
- Santos, I. R., Eyre, B. D., and Glud, R. N.: Influence of porewater advection on denitrification in permeable carbonate sediments: Evidence from flow trough reactor experiments, *Geochim. Cosmochim. Acta.*, 96, 247–258, 2012a.
- Santos, I. R., Eyre, B. D., and Huettel, M.: The driving forces of pore water and groundwater flow in permeable coastal sediments, *Estuar. Coast. Shelf Sci.*, 98, 1–15, 2012b.
- Seitzinger, S. P. and D’Elia, C. F.: Preliminary studies of denitrification on a coral reef, in: *The ecology of coral reefs*, Symposia series for undersea research, edited by: Reaka, M. L., National Oceanic Atmospheric Administration: Department of Commerce, Washington, DC, 194–208, 1985.
- Seitzinger, S. and Giblin, A. E.: Estimating denitrification in North Atlantic continental shelf sediments, *Biogeochemistry*, 35, 235–260, 1996.
- Seitzinger, S., Harrison, J. A., Bohlke, J. K., Bouwman, A. F., Lowrance, R., Peterson, B., Tobias, C., and Van Drecht, G.: Denitrification across landscapes and waterscapes: a synthesis, *Ecol. App.*, 16, 2064–2090, 2006.
- Trimmer, M. and Nicholls, J. C.: Production of nitrogen gas via anammox and denitrification in intact sediment cores along a continental shelf to slope transect in the north Atlantic, *Limnol. Oceanogr.*, 54, 577–589, 2009.
- Tupas, L. and Koike, I.: Simultaneous uptake and regeneration of ammonium by mixed assemblages of heterotrophic marine bacteria, *Mar. Ecol. Prog. Ser.*, 70, 273–282, 1991.
- Van Duyl, F. C., Van Raaphorst, W., and Kop, A. J.: Benthic bacterial production and nutrient sediment-water exchange in sandy North Sea sediments, *Mar. Ecol. Prog. Ser.*, 100, 85–95, 1993.
- West, B. T., Welch, K. B., and Galecki, A. T.: *Linear mixed models: A practical guide using statistical software*, Chapman and Hall/CRC Taylor & Francis Group, 2006, Boca Raton, Florida, USA.
- Wild, C., Tollrian, R., and Huettel, M.: Rapid recycling of coral mass-spawning products in permeable reef sediments, *Mar. Ecol. Prog. Ser.*, 271, 159–166, 2004.
- Williams, S. L., Gill, I. P., and Yarish, S. M.: Nitrogen cycling in backreef sediments, in: *Proceedings of the 5th International Coral Reef Congress*, Tahiti, 27 May–1 June, 389–394, 1995.
- Wyatt, A. S. J., Lowe, R. J., Humphries, S., and Waite, A. M.: Particulate nutrient fluxes over a fringing coral reef: relevant scales of phytoplankton production and mechanisms of supply, *Mar. Ecol. Prog. Ser.*, 406, 113–136, 2010.