Seasonal, daily and diel N₂ effluxes in permeable carbonate sediments

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Abstract. Benthic metabolism and inorganic nitrogen and N₂ flux rates (denitrification) were measured in permeable carbonate sands from Heron Island (Great Barrier Reef). Some of the N₂ 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 metabolism and inorganic nitrogen and N₂ flux rates (denitrification) were measured in permeable carbonate sands from Heron Island (Great Barrier Reef). Some of the N₂ 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 phytosynthetically active radiation reaching the sediments. Dark N₂ 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 N₂ 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 N₂ 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 Draxler, 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 C₂H₂ 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 μmol N₂-N m⁻² h⁻¹) most likely reflects the C₂H₂ blockage technique underestimating coupled nitrification–
denitrification due to inhibition of nitrification (Hynes and
Knowles, 1978). More recently higher direct N₂ fluxes (3
to 94 μmol N₂-N m⁻² 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 car-
bonate sediments (Eyre et al., 2008). As the advective flow in
benthic chambers increased, the N₂ 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
denitrification with a rapid increase in N₂ effluxes in re-
spose to the episodic deposition of phytodetritus associated
with coral mass spawning (Eyre et al., 2008). Measured den-
itrification rates with advection were also much higher (34
to 480 μmol N₂-N m⁻² 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 sea-
on (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 tempo-
rally in response to controlling factors such as carbon and ni-
trogen 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 sum-
mer, 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 N₂ fluxes (den-
itrification). 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 car-
bonate 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 shore-
line 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 × 10⁻¹⁰ m² and a porosity of
44 %; the very coarse sands had an average grain size of 1.160
microns, a permeability of 0.6 × 10⁻¹⁰ m² 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 (Huet-
tel 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 L m⁻² 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 L m⁻² d⁻¹; average
150 L m⁻² d⁻¹; Santos et al., 2010), these higher rates in-
clude all advective processes (e.g. tidal pumping), not just the
small-scale advective processes in the sediments (e.g. topog-
raphy induced advection). Most importantly, it was the same
stirring rate that was used for most of the benthic metabolism
denitrification measurements from previous studies at
the same site (i.e. Glud et al., 2008; Eyre et al., 2008), allow-
ing 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 carbon-
ate 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 phytosynthetically ac-
tive radiation (PAR) was measured every 30 min and wa-
ter depth every hour. HOBO Lux units were converted to
μE m⁻² s⁻¹ by multiplying by 0.0165 and then calibrated
against a Li-Cor-192 light meter. All the benthic incuba-
tions were undertaken over a 20.00 h diel cycle. The cham-
bers were then capped at 21.00 h, and the first samples from
the chambers were collected. Subsequent samples were col-
clected at 2.00 h, 5.00 h (dawn), 12.00 h and 17.00 h (dusk).
N₂ : 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 con-
founded 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 col-
umn (Glud et al., 2008; Cyronak et al., 2013). During the
Table 1. Summary of benthic chamber sampling campaigns.

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

summer campaign the chambers were moved between 17.00 and 18.00 h within a 100 m² 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, N₂: 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. N₂: 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. N₂: Ar samples were poisoned with 20 µL of 5 % HgCl₂ and stored submerged at ambient temperature. Details of the nutrient and N₂: Ar analysis are given in Eyre and Ferguson (2005). Dissolved oxygen measurements (±0.01 mg L⁻¹) 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 h to 17.00 h. N₂ light flux rates were calculated using concentration data from 5.00 h to 8.00 h:

- gross benthic oxygen production (GPP) = light O₂ flux (NPP) − dark O₂ flux (respiration);
- benthic O₂ p/r = GPP × daylight hours/respiration × 24 h;
- and it was assumed that the phytosynthetic quotient (PQ) and respiratory quotient (RQ) = 1.

Careful consideration was given to the possible effect of bubbles on N₂ fluxes (Eyre et al., 2002). All samples over 96 % O₂ saturated were excluded from N₂ 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 % O₂ 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 N₂ rates were calculated using concentration data from 2.00 to 5.00 h. Bubbles have the effect of reducing the first dark sample N₂ concentration, giving much higher dark N₂ flux rates, and reducing the last light sample N₂ concentration, giving lower light N₂ flux rates or apparent rates of N-fixation. Many net N₂ fluxes are also missing as they could only be calculated when light fluxes were available. Because N₂ fluxes were measured, they include both canonical denitrification and anammox. The terms N₂ 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...
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. \( \text{NH}_4^+ \), \( \text{NO}_3^- \), \( \text{O}_2 \), and \( \text{N}_2 \)) and treatment (i.e. dark, light and net for all fluxes and also net primary production (NPP)), gross primary production (GPP) and \( \text{GPP}/\text{respiration} \) \((p/r)\) 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 \( \text{NO}_3^- \) 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 \( \text{O}_2 \) (respiration), gross

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

<table>
<thead>
<tr>
<th>Flux Type</th>
<th>Season</th>
<th>Sediment Type</th>
<th>Season × Sediment Type</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dark NH(_4^+)</strong></td>
<td>( F = 4.593_{3.62} ) ( p = 0.006 ) ((\text{Summer &lt; winter, late summer, spring}))</td>
<td>( F = 0.011_{1.62} ) ( p = 0.911 )</td>
<td>( F = 0.079_{1.62} ) ( p = 0.780 )</td>
</tr>
<tr>
<td><strong>Light NH(_4^+)</strong></td>
<td>( F = 1.558_{3.63} ) ( p = 0.208 ) ((\text{Summer &lt; late, summer &lt; spring, winter}))</td>
<td>( F = 0.730_{1.63} ) ( p = 0.396 )</td>
<td>( F = 0.707_{1.63} ) ( p = 0.404 )</td>
</tr>
<tr>
<td><strong>Net NH(_4^+)</strong></td>
<td>( F = 6.996_{3.62} ) ( p &lt; 0.001 ) ((\text{Summer &lt; winter, late summer, spring}))</td>
<td>( F = 0.366_{1.62} ) ( p = 0.547 )</td>
<td>( F = 1.606_{1.62} ) ( p = 0.210 )</td>
</tr>
<tr>
<td><strong>Dark NO(_3^-)</strong></td>
<td>( F = 39.202_{3.62} ) ( p &lt; 0.001 ) ((\text{Spring &lt; winter}))</td>
<td>( F = 10.654_{1.62} ) ( p = 0.002 )</td>
<td>( F = 4.296_{1.62} ) ( p = 0.042 )</td>
</tr>
<tr>
<td><strong>Light NO(_3^-)</strong></td>
<td>( F = 4.040_{3.64} ) ( p = 0.011 ) ((\text{Spring &lt; winter}))</td>
<td>( F = 10.293_{1.64} ) ( p = 0.002 )</td>
<td>( F = 1.000_{1.64} ) ( p = 0.321 )</td>
</tr>
<tr>
<td><strong>Net NO(_3^-)</strong></td>
<td>( F = 20.076_{3.64} ) ( p &lt; 0.001 ) ((\text{Summer &lt; Late Summer, Spring &lt; Winter}))</td>
<td>( F = 20.540_{1.64} ) ( p &lt; 0.001 )</td>
<td>( F = 2.792_{1.64} ) ( p = 0.100 )</td>
</tr>
<tr>
<td><strong>Dark DO</strong> ( (\text{respiration}) )</td>
<td>( F = 18.408_{3.62} ) ( p &lt; 0.001 ) ((\text{Summer, late Summer &lt; spring, winter}))</td>
<td>( F = 0.005_{1.62} ) ( p = 0.944 )</td>
<td>( F = 9.922_{1.62} ) ( p = 0.003 )</td>
</tr>
<tr>
<td><strong>Light DO</strong> ( (\text{NPP}) )</td>
<td>( F = 68.988_{3.63} ) ( p &lt; 0.001 ) ((\text{Spring &lt; winter, late summer}))</td>
<td>( F = 0.444_{1.63} ) ( p = 0.508 )</td>
<td>( F = 3.917_{1.63} ) ( p = 0.052 )</td>
</tr>
<tr>
<td><strong>GPP</strong></td>
<td>( F = 57.030_{3.63} ) ( p &lt; 0.001 ) ((\text{Spring, winter &lt; summer, late summer}))</td>
<td>( F = 0.629_{1.63} ) ( p = 0.431 )</td>
<td>( F = 5.373_{1.63} ) ( p &lt; 0.024 )</td>
</tr>
<tr>
<td><strong>P/r DO</strong></td>
<td>( F = 22.276_{3.62} ) ( p &lt; 0.001 ) ((\text{Spring, winter &lt; summer, late summer}))</td>
<td>( F = 2.042_{1.62} ) ( p = 0.158 )</td>
<td>( F = 1.242_{2.62} ) ( p = 0.296 )</td>
</tr>
<tr>
<td><strong>Dark N(_2)</strong></td>
<td>( F = 6.164_{3.55} ) ( p = 0.001 ) ((\text{Winter, spring, summer &lt; late summer}))</td>
<td>( F = 1.692_{1.55} ) ( p = 0.199 )</td>
<td>( F = 0.414_{1.55} ) ( p = 0.523 )</td>
</tr>
<tr>
<td><strong>Light N(_2)</strong></td>
<td>( F = 3.108_{3.23} ) ( p = 0.046 ) ((\text{Summer, winter, late summer &lt; spring}))</td>
<td>( F = 0.299_{1.23} ) ( p = 0.590 )</td>
<td>–</td>
</tr>
<tr>
<td><strong>Net N(_2)</strong></td>
<td>( F = 2.157_{3.19} ) ( p = 0.127 )</td>
<td>( F = 0.000_{1.19} ) ( p = 0.986 )</td>
<td>–</td>
</tr>
</tbody>
</table>
benthic primary production (GPP) and dark \( NO_3^- \) (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 \( NO_3^- \) 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 \( O_2 \) in the dark (respiration) and produced \( O_2 \) in the light (NPP) (Fig. 1). \( NH_4^+ \) and \( NO_3^- \) fluxes generally showed uptakes in the dark and smaller uptakes or effluxes in the light (Figs. 2 and 3). \( N_2 \) effluxes were mostly reduced in the light (Fig. 4).

All benthic fluxes showed a significant difference between seasons, except light \( NH_4^+ \), light \( NO_3^- \) and net \( N_2 \) fluxes (Table 2). Dark \( O_2 \) uptakes (respiration), GPP, NPP and GPP/dark \( O_2 \) \( (p/r) \) ratios were all higher in late summer and summer than the other seasons (Fig. 1). \( NH_4^+ \) and \( NO_3^- \) fluxes all showed greater uptakes in summer than the other seasons (Figs. 2 and 3). Dark \( N_2 \) effluxes were highest in late summer, but light \( N_2 \) 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 \( \mu \text{E m}^{-2} \text{s}^{-1} \)) and 7 (1717 \( \mu \text{E m}^{-2} \text{s}^{-1} \)) when the water depth was shallowest.

### Table 3. Results from paired \( t \) test to determine differences in dark and light fluxes.

<table>
<thead>
<tr>
<th>( \text{Parameter} )</th>
<th>( \text{NH}_4^+ )</th>
<th>( \text{NO}_3^- )</th>
<th>( \text{DO} )</th>
<th>( \text{N}_2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( t )</td>
<td>3.605</td>
<td>9.057</td>
<td>32.173</td>
<td>11.961</td>
</tr>
<tr>
<td>( p )</td>
<td>0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>( \text{Difference} )</td>
<td>( \text{Dark} &lt; \text{Light} )</td>
<td>( \text{Dark} &lt; \text{Light} )</td>
<td>( \text{Dark} &lt; \text{Light} )</td>
<td>( \text{Light} &lt; \text{Dark} )</td>
</tr>
</tbody>
</table>

\( p/r \) ratios \( (p < 0.001) \) and dark \( NH_4^+ \) 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 \( NH_4^+ \) uptakes were greater on days 5–7 (post-spawn) than days 1–4 (pre-spawn). The dark \( NH_4^+ \) uptakes peaked on day 10 and then decreased to day 12.

Dark \( O_2 \) 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 \( NO_3^- \) 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 \( N_2 \) fluxes over the first 7-day period \( (p = 0.086) \), but dark \( N_2 \) 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 \( C_2H_2 \) 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 \( C_2H_2 \) 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 \( C_2H_2 \) 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 \( N_2 \) flux technique in carbonate 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.

<table>
<thead>
<tr>
<th>Rate (µmol N₂•m⁻²•h⁻¹)</th>
<th>System</th>
<th>Season</th>
<th>Method</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>19 (dark) Sands, Bahamas</td>
<td>Dark, C₂H₂, slurry</td>
<td>D’Elia (1985)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.12 to 0.45 (dark) Sands, Hopkinson Reef, GBR</td>
<td>Dark, C₂H₂, slurry</td>
<td>Capone et al. (1992)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.2 to 13.3 (dark) Sands, Bowl Reef, GBR</td>
<td>Dark, C₂H₂, slurry</td>
<td>Capone et al. (1992)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.7 to 6.5 (dark) Sands, Ishigaki Island</td>
<td>Dark, C₂H₂, slurry</td>
<td>Miyajima et al. (2001)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 to 37 (dark) Carbonate muds, Arlington Reef, GBR</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7 to 83 (dark) Carbonate muds, Sudbury Reef, GBR</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2006)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 to 67 (dark) Fine to medium inter-reef channel sands</td>
<td>Winter</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>61 to 94 (dark) Fine to medium inter-reef channel sands</td>
<td>Summer</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>60 to 85 (dark) Very coarse outer reef channel sands</td>
<td>Spring</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>19 to 65 (dark) Very coarse outer reef channel sands</td>
<td>Summer</td>
<td>Dark, Direct N₂, ex situ chamber, no advective flow</td>
<td>Alongi et al. (2011)</td>
<td></td>
</tr>
<tr>
<td>34 to 92 (dark) Pre-spawning, very coarse permeable sands, Heron Island, GBR</td>
<td>Summer</td>
<td>Dark, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>Eyre et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>96 to 152 (dark) 1–3 days post-spawning, very coarse permeable sands, Heron Island, GBR</td>
<td>Summer</td>
<td>Dark, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>Eyre et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>400 to 480 (dark) 4–5 days post-spawning, very coarse permeable sands, Heron Island, GBR</td>
<td>Summer</td>
<td>Dark, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>Eyre et al. (2008)</td>
<td></td>
</tr>
<tr>
<td>54 (dark) Very coarse permeable sands, Heron Island, GBR</td>
<td>Winter</td>
<td>Dark, light, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>66 (light)</td>
<td>Winter</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60 (net)</td>
<td>Winter</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80–214 (dark) Very coarse permeable sands, Heron Island, GBR</td>
<td>Summer</td>
<td>Dark, light, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>2–14 (light)</td>
<td>Summer</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7–70 (net)</td>
<td>Summer</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>234 (dark) Very coarse permeable sands, Heron Island, GBR</td>
<td>Late Summer</td>
<td>Dark, light, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>52 (light)</td>
<td>Late Summer</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>128 (net)</td>
<td>Late Summer</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>82 (dark) Very coarse sands, Heron Island, GBR</td>
<td>Spring</td>
<td>Dark, light, N₂ : Ar, in situ chamber, advective flow, 40 rpm</td>
<td>This study</td>
<td></td>
</tr>
<tr>
<td>344 (light)</td>
<td>Spring</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
<tr>
<td>234 (net)</td>
<td>Spring</td>
<td>This study</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
denitrification rates of a similar range to those measured in Eyre et al. (2008) and this study (60 to 304 µmol N m$^{-2}$ h$^{-1}$) 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 (µmol O$_2$ m$^{-2}$ h$^{-1}$) and dark N$_2$ efflux (µmol N$_2$-N m$^{-2}$ h$^{-1}$). Regressions were forced through zero as there would be no N$_2$ efflux when respiration equals zero and to allow comparisons with previous studies. Outliers were removed from all regressions (see Discussion).

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

1 Data from Fig. 4 in Glud et al. (2008) and Fig. 3 in Eyre et al. (2008); 2 Data from sites KR, PR, MR, OB and IR in Tables 4 and 8 in Alongi et al. (2008); 3 Alongi et al. (2008) and data from site PRC4 and OS1B in Table 3 and Fig. 8 in Alongi et al. (2011); 4 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.

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$_4^+$ 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$_4^+$ 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 ($r^2 =$...
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 \( \text{NH}_4^+ \) uptakes and NPP are positively correlated \( (r^2 = 0.55; \ p \leq 0.01; \ n = 26) \), reflecting the dark uptake of \( \text{NH}_4^+ \) by benthic microalgae. A clear pattern of \( \text{NH}_4^+ \) uptake in the light is not seen because water column \( \text{NH}_4^+ \) 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 \( \text{NH}_4^+ \) 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 \( \text{NH}_4^+ \) 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 \( \text{N}_2 \) fluxes were strongly controlled by benthic respiration (Fig. 5; Table 5), most likely reflecting an increased supply of \( \text{NH}_4^+ \) from ammonification for coupled nitrification–denitrification and the availability of electron donors for denitrification. \( \text{NO}_3^- \) uptakes were not significantly correlated with \( \text{N}_2 \) fluxes due to the very low water column \( \text{NO}_3^- \) concentrations \( (< 1 \mu \text{mol L}^{-1}; \ \text{Eyre et al., 2008}) \), 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 \( \text{N}_2 \) 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 (NO$_3^-$) fluxes (mean ± 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$_2$ 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$_2$ efflux (Fig. 6; Table 5). Despite improving the relationship, the inter-reef carbonate sands generally had lower rates of benthic respiration and associated N$_2$ 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 was 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$_2$ 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$_2$ efflux due to competition from benthic microalgae is given by the
negative correlation between dark N$_2$ efflux and dark NH$_4^+$ ($r^2 = 0.34$, $p = 0.05$, $n = 29$) and NO$_3^-$ ($r^2 = 0.24$, not significant, $n = 29$) uptakes, and dark N$_2$ 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$_2$ 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 Duyl et al., 1993; Lomstein et al., 1998). Several types of bacteria can assimilate NH$_4^+$, including sulphate reducers and fermentative bacteria (Koike and Sumi, 1989). Additionally, sulphate reducers can fix nitrogen (Nielsen et al., 2001). N$_2$ effluxes are a measure of denitrification minus N-fixation; as such, increased N-fixation will result in a reduced N$_2$ efflux. Further, coupled nitrification–denitrification may be suppressed by H$_2$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 ($O_2$ respiration/(N$_2$-N+NH$_4^+$+NO$_3^-$)) 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$_2$ and associated leakage of NO$_2$ that fuels anammox.
(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 N₂ production for a given benthic
respiration, not less (i.e. lower slope).

Further support for the low slope of the relationship be-
tween benthic respiration and dark N₂ 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 relation-
ship in tropical carbonate sands (Fig. 6). The outliers fall
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 depo-
sition 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 phy-
todetritus, and no heterotrophic assimilation of nitrogen
due to low C:N. In addition, flow-through reactor experi-
ments 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 N₂ 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 ((N₂-N/Dark O₂
Flux/6.6) × 100 %) with a maximum loss of about 15 % or
less as N₂ of the remineralised nitrogen for Redfield organic
matter (Cardenas et al., 2008; Kessler et al., 2012). Using the
benthic respiration versus dark N₂ slope of 0.035 for tropical
carbonate sands gives losses of 23, 42 and 71 % as N₂ of the
remineralised nitrogen for organic matter C:N ratios
between benthic respiration and dark N₂ efflux 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 N₂ production for a given benthic
respiration, not less (i.e. lower slope).

Further support for the low slope of the relationship be-
tween benthic respiration and dark N₂ 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 relation-
ship in tropical carbonate sands (Fig. 6). The outliers fall
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 depo-
sition 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 phy-
todetritus. The remineralisation ratios ($O₂$ respiration/ ($N₂-
N+NH₄⁺ + NO₃⁻)) 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 experi-
ments 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 N₂ efflux
slope of 0.114 (Santos et al., 2012a), which is also similar to
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Modelling and flume studies have suggested that
permeable quartz sands have a low respiration–
nitrification–denitrification efficiency ((N₂-N/Dark O₂
Flux/6.6) × 100 %) with a maximum loss of about 15 % or

\[ \text{Slope} = 0.035 \]
\[ r^2 = 0.63 \]
\[ p < 0.005 \]

\[ \text{Slope} = 0.089 \pm 0.009 \]
\[ r^2 = 0.82 \]
\[ p < 0.005 \]

\[ \text{Slope} = 0.035 \pm 0.001 \]
\[ r^2 = 0.71 \]
\[ p < 0.005 \]

This Study  Eyre et al. 2008  Alongi et al. 2008  Alongi et al. 2011

Fig. 5. Dark N₂ effluxes versus benthic respiration for all seasons
in very coarse and coarse sands (individual chambers).

Fig. 6. Dark N₂ effluxes versus benthic respiration for carbonate
sands from this study (individual chambers), Eyre et al. (2008) (in-
dividual chambers) and Alongi et al. (2008, 2011). See Table 4 for
sites used from Alongi et al. (2008, 2011). Open symbols are out-
liers that fall above the upper 95% confidence interval of the regres-
sions of each of the 3 data sets above (see Discussion).
at high respiration rates (> 5000 µmol m\(^{-2}\) h\(^{-1}\)). In contrast, the denitrification efficiency of muds decreases rapidly above respiration rates of around 1500 µmol m\(^{-2}\) 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\(_2\) fluxes are generally much lower than dark N\(_2\) effluxes probably due to N-fixation by cyanobacteria in the microphytobenthos. Alternatively, competition for nitrogen with benthic microalgal 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\(_2\) fluxes. The resultant net N\(_2\) fluxes would result in far less nitrogen loss from the system than would be estimated using dark N\(_2\) 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\(_2\) sinks with N-fixation in the light exceeding denitrification in the dark, but this cannot be determined using the N\(_2\)/Ar method. Unfortunately, bubbles are produced during the light in the highly productive sediments, which reduces the N\(_2\)/Ar ratio (see Discussion in Eyre et al., 2002), giving a pseudo-rate of N-fixation. As such, most of the light N\(_2\)/Ar data in this study, and all of the light N\(_2\)/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 µmol N\(_2\)-N m\(^{-2}\) h\(^{-1}\) reviewed in Capone et al., 1992), including at this site (< 37.4 µmol N\(_2\)-N m\(^{-2}\) 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 Droxl, 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 Tg N yr\(^{-1}\) (Seitzinger et al., 2006) was based on a slope of 0.116 for benthic respiration and dark N\(_2\) (Table 5; Seitzinger and Giblin, 1996) and is within the range of previous estimates (214–300 Tg N yr\(^{-1}\); reviewed in Seitzinger et al., 2006). If the lower slope (0.035) for benthic respiration and dark N\(_2\) (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 with 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\(^{-1}\), 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, \(N\) 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 towards better characterising the relationship between benthic respiration and dark and light \((\text{net})\) \(N\) efflux for permeable carbonate and quartz sands. In particular, the interaction between diel cycles in benthic metabolism and denitrification, \(N\)-fixation, and net \(N\) effluxes and advective porewater flow should be investigated.

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