Sources of nutrients driving production in the Gulf of Carpentaria, Australia – a shallow tropical shelf system

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Running header: Nitrogen processes in a tropical shelf system

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2
3 **Abstract**

4 The tropical Gulf of Carpentaria, Australia has recently been identified as one of the
5 world's least impacted marine areas presenting a unique opportunity to understand the
6 nutrient drivers of productivity. This study examined the nitrogen (N) sources and
7 transformations in this pristine area, and its' role in fuelling primary productivity
8 principally based on summer data. The nitrogen budget estimates on a whole-of-Gulf
9 basis suggest that river nitrogen inputs are unlikely to be major contributors to
10 primary productivity. In the deeper waters of the Gulf, beyond the coastal boundary
11 current, the main source of nitrogen is estimated to be nitrogen fixation by
12 cyanobacteria, principally the abundant genus, *Trichodesmium*. Our study measured
13 high nitrogen fixation rates and depleted $\delta^{15}\text{N}$ -nitrogen ratios in the particulate matter
14 in the water column during a summer bloom. During summer, bottom nitrogen
15 concentrations increased and $\delta^{15}\text{N}$ -nitrogen ratios were depleted, suggesting that
16 benthic mineralization is occurring. It is therefore likely that detrital material from
17 nitrogen-rich *Trichodesmium* is an important contributor to benthic processes. During
18 winter, wind-driven mixing results in nitrogen from bottom waters reaching the
19 euphotic zone, and fuelling primary productivity. Therefore, *Trichodesmium* has an
20 important direct and indirect role in contributing to primary productivity in this
21 pristine tropical ecosystem.

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23 Additional keywords: nitrogen fixation, *Trichodesmium*, nitrogen budget

Introduction

Tropical coastal waters are characterized by high water temperatures and large summer rainfall events resulting in significant river inflows and associated inputs of nutrients from adjacent rivers. This contributes to high year-round primary production (Longhurst and Pauly 1987). Development of catchments for agriculture and other human activities has typically reduced or regulated river flows in many rivers, and increased nutrient loads to coastal waters (Carpenter *et al.* 1998; Beman *et al.* 2005). The increase in algal blooms worldwide, including harmful species, and development of dead zones in coastal waters has been attributed to increasing nutrient inputs from catchments (Ferber 2001; Anderson *et al.* 2002; Chen *et al.* 2007).

Further offshore, many areas of the tropics have low dissolved inorganic nitrogen (N) concentrations and low N:P ratios, resulting in N limitation. In these areas, atmospheric N inputs from cyanobacteria can be an important source of N. The most studied species is the filamentous diazotroph *Trichodesmium* which can form large surface rafts stretching hundreds of kilometres, adding N to fuel productivity (Westberry and Siegal 2006). More recently, the importance of other symbiotic and small unicellular cyanobacteria in providing N to tropical food webs has also been established (Carpenter *et al.* 1999; Zehr *et al.* 2001; Montoya *et al.* 2004; Capone *et al.* 2006).

The Gulf of Carpentaria in tropical Australia is a large coastal sea (330,000 km²) surrounded by many large rivers seasonally providing 92,000 GL of water to the Gulf each year during the NW monsoonal wet season (January to March). The shallow depth of the Gulf (<70 m) combined with the high tidal energy means that tidal and wind mixing dominates over advective processes (Forbes 1984; Wolanski and Ridd 1990). There is also relatively little mixing of waters in the Gulf with the adjacent Arafura and Coral Seas (Forbes 1984; Condie and Dunn 2006). Within the Gulf there are two distinct regions: a turbid nearshore zone at a depth of 15 to 20 m; and deeper waters separated from the coastal zone by a boundary (buoyancy) current (Wolanski and Ridd 1990). River runoff is effectively trapped within the coastal zone.

Unlike many tropical areas around the world, this region has a low human population (<10 people km²) and limited human activities in the seas or catchments. It has been identified as one of the least anthropogenically impacted areas globally (Halpern *et al.* 2008). However, there is increasing pressure for human development in the Gulf region, including water extraction for irrigation, and regulation of water flow by damming. However, the role of river nutrient inputs in promoting coastal production, and their links with offshore production, remain poorly understood. In light of this, this study examined the relative contribution of river N inputs versus N fixation by *Trichodesmium* by developing a N budget for the Gulf, based on measurements in this study and historical data.

Materials and Methods

Study Location

Three separate sampling trips to the Gulf of Carpentaria in the summer wet season were undertaken. Voyage 1 was from 12 to 29 January 2004 and the areas sampled were: the offshore waters adjacent to Albatross Bay (15 – 30 m deep); the southeastern Gulf near Karumba (10 – 25 m deep); near Mornington Is. (35 – 40 m deep); the Vanderlins (40 – 50 m deep); and Groote Eylandt (35 – 40 m deep) (Fig. 1, Table 1). Voyage 2 was on 8 February 2005 and Albatross Bay (7 – 10 m deep), which is within the coastal boundary layer, was sampled. Voyage 3 was between 27 February and 19 March 2005 and three offshore areas adjacent to Mornington Island (35 – 40 m deep), the Vanderlins (40 – 50 m deep) and Groote Eylandt (35 – 40 m deep) were sampled.

The first two trips focussed on sampling the water column and sediment particulate organic matter for stable isotope analyses (Table 1). Additionally, on 8 February 2005, infaunal samples were also taken in Albatross Bay. In the final trip, physico-chemical parameters (temperature, oxygen, dissolved inorganic nutrients, chlorophyll *a*) were sampled through the water column. Samples of particulate organic matter were also taken in the water column, sediment for stable isotope analyses and infaunal stable isotope analysis. Sediment box cores were also collected for denitrification measurements. There was a bloom of the cyanobacterium *Trichodesmium* during the voyage and N fixation incubations were done.

Nutrients and physico-chemical parameters

On voyage 3 (Feb-Mar 2005) water samples were collected every 10 m through the water column at 123 stations for nutrient analyses. Chlorophyll *a* samples were collected at 9 stations. A 12-bottle rosette of 10-L Niskin bottles was used to collect water. The samples were immediately put into glass and plastic bottles. Temperature and oxygen were measured continuously through the water column using a conductivity-temperature-depth (CTD) logger. At the commencement of the voyage the water column was thermally stratified, however, mid-way through the voyage a cyclone passed through the sampling area causing the water column to be completely mixed for much of the remainder of the voyage. As a result it is not valid to compare nutrient, chlorophyll *a* and other physico-chemical parameters between stations, regions and depths.

Silicate, phosphate and nitrate plus nitrite concentrations were measured on board with an autoanalyzer during the Gulf transects (Airey and Sandars 1987). The nitrate method was modified with imidazole buffer and cleaned cadmium granules to convert nitrate to nitrite. Ammonium concentrations were measured on frozen samples on shore using the standard colorimetric methods (American Public Health Association 1995).

For chlorophyll analysis, known volumes of water from the surface and bottom were filtered through glass fibre filters (Whatman GF/F) to trap the particulates. The filters were then frozen until returned to the laboratory. Filters were extracted by probe sonicating for 1 min in cold 100% acetone, and extracts were measured spectrophotometrically after adjusting the acetone concentration to 90%, when there was sufficient chlorophyll, and with a spectrofluorometer when chlorophyll values were low (Jeffrey and Welshmeyer 1997).

N fixation measurements

Concentrated *Trichodesmium* trichomes were observed in the surface waters on 2 March 2005. A bucket was used to sample the surface water. Subsamples were poured to overflowing into twelve 250-mL Schott bottles with sealed rubber ports in the lid. Acetylene gas, pre-bubbled through water to remove contaminants, was bubbled into the sample in the sealed bottles for 5 min to ensure saturation of the water with acetylene. Triplicate bottles were incubated at four light levels using shade bags: 100%,

50%, 8% and 0% surface light. Samples were incubated on deck for 24 h in a plastic tub with flow-through seawater taken near the surface. Temperature was logged throughout the incubations in an additional bottle using a sensor (ETemperature, On Solutions, Baulkham Hills, Australia). Water subsamples (3 mL) were withdrawn from the bottles into Venoject vials containing 0.2 mL 20% trichloroacetic acid to halt N fixation at 0, 4 and 24 h. Subsamples were stored at 4°C until analyzed in the laboratory. Gas from the headspace of the vials was injected into a gas chromatograph with a flame ionization detector to determine the ethylene content of the samples. N fixation rates were calculated from the conversion of acetylene to ethylene (Knowles 1990).

Subsamples of water were taken at time zero (t_0) for chlorophyll *a* concentrations, algal cell counts and ^{15}N -nitrogen stable isotope ratios. For chlorophyll analysis, the method described above was used. For *Trichodesmium* cell counts, samples were fixed in 1% glutaraldehyde and kept cool until enumerated using a Sedgwick Rafter counting chamber and a compound microscope (Leica) under 400 x magnification. For stable isotope analyses of *Trichodesmium*, triplicate water samples were filtered through precombusted glass fibre filters (Whatman GF/F) to trap the particulate organic matter. Filters were then frozen until returned to the laboratory. Filters were dried at 60°C in an oven then analyzed for $\delta^{15}\text{N}$ -nitrogen ratios with a continuous-flow isotope-ratio mass spectrometer (Europa Tracermass and Roboprep, Crewe, England).

Denitrification measurements

Rates of denitrification were measured in the sediment using the isotope pairing technique (Nielsen 1992) as described by Dalsgaard *et al.* (2000). Box cores were taken at 29 sites across the three regions. Four sub-cores (4.8 cm id $\times$ 30 cm) were taken from each site such that there was *ca.* 8 cm of sediment (and would be *ca.* 17 cm of water column during incubation).

A teflon-coated stirrer bar was then suspended ~5 cm above the sediment. This was driven by an external rotating magnet rotating at 60–70 rpm. Typically core tubes from three consecutive sites were collected and incubated for each batch. Incubation bins were filled with seawater such that the cores were immersed, and allowed to

equilibrate for 6 h or more. After equilibration, the water level was reduced to below core tube height and experiments commenced with the addition of stock ^{15}N -nitrate to a concentration of 60 μM . This concentration was assumed to be a saturating concentration based on studies by Cook *et al.* (2004). The water in each core was stirred for 20 min and sub-samples taken for the analysis of nitrate in order to calculate the final ^{15}N enrichment. Cores were then capped with sealed Perspex lids and incubated in the dark in flowing seawater. Cores were sacrificed at 0, 1.4, 2.5, 4.5 and 6 h. At each time point, 1 mL of 50% zinc chloride was added to the water overlying the sediment before the sediment was gently slurried with the water column using a metal rod, coarser particles were allowed to settle for about a minute before a subsample was taken using a gas tight syringe. The sample was then placed in a 12.5 mL Exetainer (Labco, High Wycombe, UK) to which 250 μL of 50% w/v zinc chloride had been added and stored at 4°C until returned to the laboratory.

A headspace of helium was introduced into the Exetainer within 2 weeks and the samples were then analyzed within several months. Headspace analysis for $^{28}\text{N}_2$ -, $^{29}\text{N}_2$ - and $^{30}\text{N}_2$ -nitrogen gas was carried out by removing a 50 μL sample in a gas tight syringe and injecting into a gas chromatograph (5890 Hewlett Packard) in line with an isotope ratio mass spectrometer (Finnigan MAT delta S). A copper reduction column heated to 640°C was used to remove oxygen from the sample, carbon dioxide and water were removed using a liquid N cryotrap. Denitrification rates were calculated according to the isotope pairing equations in Dalsgaard *et al.* (2000).

Stable isotope ratios

The stable isotope ratios were determined for particulate organic matter in the Gulf of Carpentaria in January 2004 and February/March 2005. The following regions were sampled: near Karumba; near Mornington Island; the Vanderlins; Groote Eylandt, and Albatross Bay (inshore, offshore) (Fig. 1, Table 1). For stable isotope analyses in particulate material, triplicate water samples from the surface were filtered through precombusted glass fibre filters (Whatman GF/F) to trap the particulate organic matter. In the case of the third voyage, bottom water samples were also taken and filtered. Filters were then frozen until returned to the laboratory. Filters were dried at 60°C and

analyzed for $\delta^{15}\text{N}$ -nitrogen ratios with a continuous-flow isotope-ratio mass spectrometer (Europa Tracermass and Roboprep, Crewe, England).

For sediment stable isotope ratios, box cores were taken at 48 sites across three regions (Mornington Island, Vanderlins, Groote Eylandt). Three small cores (31 mm dia.) were taken from one box core at each site for measurement of ^{15}N -nitrogen stable isotope ratios. The sediment was extruded from the core to maintain the integrity of the surface and the top 10 mm sliced off the top. This was frozen until analyzed in the laboratory. Marine sediments are screened through a wire mesh screen to remove rocks and rubble, dried at 60°C and ground before being analyzed for $\delta^{15}\text{N}$ -nitrogen ratios with a continuous-flow isotope-ratio mass spectrometer (Europa Tracermass and Roboprep, Crewe, England).

Grab sediment cores were taken at five sites near Karumba, Groote Eylandt and Albatross Bay offshore in January 2004. Mini-cores were inserted into the surface of the sediment and the sediment was extruded from the core to maintain the integrity of the surface and the top 1 cm sliced off the top. The sediment was processed and analyzed as above.

In February 2005, sediment grabs were also collected at four sites in Albatross Bay for analysis of the stable isotope ratios of infauna. Sediment samples were frozen until analysed. Thawed samples were sieved onto 500- μm sieves and infauna separated from remaining sediment/sand particles under a stereo-microscope. Infauna consisted of crustaceans, molluscs, small fish, and polychaete worms. Molluscs were removed from their shells prior to analysis. All samples were dried at 60°C until completely dried, then ground to a fine powder with a mortar and pestle before being analyzed for $\delta^{15}\text{N}$ -nitrogen ratios with a continuous-flow isotope-ratio mass spectrometer (Europa Tracermass and Roboprep, Crewe, England). Mean ^{13}C -carbon and ^{15}N -nitrogen ratios were determined.

In February/March 2005, three sediment box cores were also taken in each of three regions, Mornington Island, Vanderlins, and Groote Eylandt, for analysis of the stable isotope ratios for infauna. Whole box core samples were sieved onto a 500 μm sieve

with water, and the trapped organisms stored frozen until analysed. In the laboratory, samples were thawed, and infauna separated from remaining sediment/sand particles. Analysis was conducted as described above and mean ^{13}C -carbon and ^{15}N -nitrogen ratios for each region were determined.

N budget

The results of this study were combined with historical data on the oceanography of the Gulf of Carpentaria to generate a N budget for the Gulf of Carpentaria. The area of the Gulf was assumed to be 330,000 km², with an average depth of 43.4 m, resulting in a volume of 1.4×10^{13} m³. The total pools of nitrate and ammonium in the water column of the Gulf were calculated based on concentrations in this as well as previous studies (Rothlisberg *et al.* 1989; Rothlisberg *et al.* 1994; Burford and Rothlisberg 1999; this study). Annual nitrate and ammonium uptake rates by phytoplankton were determined using the ^{15}N -nitrogen uptake data of Burford and Rothlisberg (1999). Annual areal N fixation by *Trichodesmium* was determined using the depth-integrated N fixation rates for a *Trichodesmium* bloom measured in this study, and assuming a bloom depth of 1 m. The calculated rates were within the range determined by Capone *et al.* (2005) for the northern Atlantic Ocean. It was assumed that *Trichodesmium* was fixing N at the calculated rates a third of a year, based on a previous study of the relative abundance of *Trichodesmium* in the Gulf (Burford *et al.* 1995).

Annual N fixation rates in the seagrass beds were determined by Moriarty *et al.* (1990). A spatial coverage of seagrass of 52,000 ha was used based on Roelofs *et al.* (2005). Annual denitrification rates were determined based on this study. River inflows were based on multiplying by total N concentrations in the water column for the Embley, Flinders, Norman and Gregory Rivers (20.7, 66.2, 24.0 and 21.7 μM N respectively, M. Burford, unpubl. data) by the annual river flow volumes (www.nrw.qld.gov.au/) for those rivers (A. Brooks, unpubl. data). For the other 25 largest rivers in the Gulf, a mean total N concentration based on the other four rivers was used. All loads were summed to give a total river N input to the Gulf.

Results

In February 2005, mean ammonium, oxides of N, phosphate and silicate concentrations did not vary across three regions (Morningson Is., Vanderlins, Groote

Eylandt) but Groote Eylandt had higher variability in nitrate concentrations than the other two sites (Fig. 2). Chlorophyll *a* concentrations were 0.80 ± 0.45 (SD) $\mu\text{g L}^{-1}$ and did not show any trends between regions.

Rate measurements

Denitrification rates measured during the study were 0.033 ± 0.054 (SD) $\mu\text{mol m}^{-2} \text{h}^{-1}$ in the Groote Eylandt region, 0.007 ± 0.010 (SD) $\mu\text{mol m}^{-2} \text{h}^{-1}$ in Mornington Is. region and 0.016 ± 0.027 (SD) $\mu\text{mol m}^{-2} \text{h}^{-1}$ in the Vanderlins region. The variability between stations was high since the rates were close to the detection limit for the method. At many stations, denitrification rates were below the detection limit.

The N fixation rate was determined during a surface *Trichodesmium* bloom (3,400 trichomes mL^{-1}) in the Mornington Island region in February 2005. N fixation rates were highest when samples were incubated at 50% surface light ($76 \mu\text{mol N m}^{-2} \text{h}^{-1}$) compared with 8% ($42 \mu\text{mol N m}^{-2} \text{h}^{-1}$) and 100% ($27 \mu\text{mol N m}^{-2} \text{h}^{-1}$) light. The depth-integrated N fixation assuming a 1 m deep surface bloom was $578 \mu\text{mol N m}^{-2} \text{d}^{-1}$. The $\delta^{15}\text{N}$ ratios of *Trichodesmium* during the bloom, and particulate matter in the water column throughout the February/March 2005 voyage were close to 0 to 1 ‰ while the molar C:N ratios were between 3 and 5 (Fig. 3). These values were compared with a previous Gulf voyage in January 2004 when no *Trichodesmium* bloom was apparent. The $\delta^{15}\text{N}$ ratio at a range of sites was substantially higher, 5 to 6.5 ‰, as was the C:N ratio (6.5 to 9).

Stable isotope ratios for sediment and infauna

The $\delta^{15}\text{N}$ ratio was also measured in the sediment during the *Trichodesmium* bloom in February/March 2005 with values ranging from 2.4 to 3.7 ‰ (Fig. 4). This compared with values ranging from 3.9 to 6.0 ‰ during two previous Gulf voyages in January 2004 and 2005 when no *Trichodesmium* blooms were apparent. Infaunal $\delta^{15}\text{N}$ ratios were compared with ratios in particulate organic matter in the water column in both a period of *Trichodesmium* bloom in the southern Gulf (February/March 2005) and a non-bloom period in the northeastern Gulf (January 2005) (Fig. 5). There were a wide range of $\delta^{15}\text{N}$ ratios for the infauna but no obvious differences between the two sampling periods. In contrast, the $\delta^{15}\text{N}$ ratios for the particulate organic matter were

substantially different between the northeast Gulf in January 2005, and the southern Gulf in February/March 2005.

Discussion

N budget

The data from this study were combined with data from previous studies in the Gulf to produce a Gulf-wide N budget (Table 3). The nitrate pool in the Gulf was higher than the ammonium pool, 75 Mt compared with 24 Mt, with nitrate taken up more rapidly by phytoplankton than ammonium (Table 3) (Rothlisberg *et al.* 1989; Rothlisberg *et al.* 1994; Burford and Rothlisberg 1999). However, much of the nitrate was present in bottom waters and therefore during periods of stratification would not be available to phytoplankton.

The importance of N fixation was determined using two methods: direct measurement of N fixation during a bloom of *Trichodesmium*, and measurement of $\delta^{15}\text{N}$ stable isotope ratios in the water column and sediment. N fixation from *Trichodesmium* was calculated to be a substantial new source of N into the Gulf added at a rate similar to nitrate and ammonium uptake (290 Mt y^{-1} compared with 390 and 260 Mt y^{-1} respectively) (Burford and Rothlisberg 1999). River loads of N were calculated to be substantially lower, i.e. 43 Mt y^{-1} , comparable with inputs of new N via N fixation in the seagrass beds. In contrast, loss of N via denitrification rates measured was low relative to inputs, i.e. 11 Mt y^{-1} . Overall N inputs to the Gulf were substantially higher than N outputs.

This study is a first attempt to determine a N budget for the Gulf of Carpentaria, Australia. Our study supports previous studies that suggest that the Gulf is N limited with rapid cycling of N (Rothlisberg *et al.* 1989; Rothlisberg *et al.* 1994; Burford and Rothlisberg 1999). Dissolved inorganic N:phosphorus ratios and dissolved inorganic N concentrations were low. Previous studies in the Gulf have also shown a response of phytoplankton to N, but not phosphorus addition (Burford and Rothlisberg 1999), and the prevalence of the N-fixing cyanobacteria, *Trichodesmium*, which typically dominates in low N environments (Burford *et al.* 1995). A study of the northwest

shelf region of Australia also showed N limitation with rapid cycling of N (Furnas and Mitchell 1999).

Additionally, this study showed that denitrification rates were low compared with other studies in coastal waters, i.e. the tropical Great Barrier Reef, the eutrophic Gulf of Gdansk and the upwelling regions off Chile (Witek *et al.* 2003; Molina *et al.* 2004; Alongi and McKinnon 2005; Alongi *et al.* 2007). These low rates may reflect low nitrate availability consistent with a N-limited system. N limitation in the Gulf parallels findings in other marine waters around Australia (Condie and Dunn 2006).

River inputs

River inputs are not major contributors of N on a whole-of-Gulf scale. However, coastal runoff is trapped within the coastal boundary layer to a water depth of approximately 20 m (Wolanski and Ridd 1990). This coastal area is approximately 20% of the area of the Gulf. In this context, river N inputs, particularly in the southern Gulf where most of the large rivers occur, may play a more important role in fuelling coastal productivity. Alongi and McKinnon (2005) found that terrestrially-derived nutrients were efficiently utilised by benthic and pelagic microbes in the coastal zone in the Great Barrier Reef shelf, in northern Australia reducing their impact offshore. It is likely that similar processes are underway in the Gulf of Carpentaria. Within the coastal boundary layer of the Gulf there is a net movement of water from east to west in the Gulf, with water ultimately entering the western Arafura Sea, rather than mixing with the central Gulf. The coastal area of the Gulf is dominated by vast highly productive seagrass beds (Moriarty *et al.* 1990; Roelofs *et al.* 2005). These provide an important food source for biota, including commercially important shrimp species (Loneragan *et al.* 1997). Estimated river N inputs were comparable with N fixation in the seagrass beds (Moriarty and O'Donohue 1993) suggesting that these inputs may provide an important source to fuel seagrass and epiphyte production.

Although N from river sources may be important to coastal production, the presence of the coastal boundary current means that it is likely that little of this source of N is likely to reach the deeper waters of the Gulf. Previous studies have not tracked riverine N offshore, but studies of $\delta^{13}\text{C}$ ratios in particulate organic matter throughout

the deeper waters of Gulf which found no evidence of a terrestrial signal (Rothlisberg *et al.* 1994; Burford, unpubl. data). Additionally, a study of food sources for estuarine shrimp in the Gulf showed no evidence that mangrove and terrestrial carbon exported from estuaries contributing to offshore food webs (Loneragan *et al.* 1997).

N fixation

This study has shown that N fixation can be a significant process in the Gulf, as demonstrated by measurement of high rates of fixation and the depleted $\delta^{15}\text{N}$ ratios in the particulate matter in the water column during a *Trichodesmium* bloom. Previous studies in the Gulf have shown the net phytoplankton community is comprised principally of the N-fixing cyanobacteria *Trichodesmium* and large diatoms (Hallegraeff and Jeffrey 1984; Rothlisberg *et al.* 1994; Burford *et al.* 1995), and that primary productivity rates are relatively high, particularly in summer months (Motoda *et al.* 1978; Rothlisberg *et al.* 1994; Burford *et al.* 1999). Previous work has also shown that chlorophyll *a* concentrations are higher in summer than winter but only in inshore regions (Burford *et al.* 1995). Further offshore, chlorophyll *a* remains similar year-round, typically less than $<1 \mu\text{g L}^{-1}$. *Trichodesmium* is numerically most dominant in the summer months when stratification occurs (Burford *et al.* 1995).

Conservative assumptions about the annual rates of N fixation from data in our study were made based on the information in seasonal abundance (Burford *et al.* 1995). Rates of N fixation measured in February/March 2005 during a *Trichodesmium* bloom ($3,400 \text{ trichomes L}^{-1}$ in surface waters) were comparable with those in other tropical waters (Capone *et al.* 2005). More recently, *Trichodesmium* has been shown to be more abundant at depth than previously thought due to sampling artefacts (Davis and McGillicuddy 2006). Therefore it is likely that rates may be significantly higher than previously thought. In contrast with some other tropical areas, the main factor likely to be limiting *Trichodesmium* growth in the Gulf of Carpentaria is iron rather than phosphorus availability (Sohm *et al.* 2006, 2008). Aeolian dust from central Australia has been shown to provide an important source of iron for diazotrophs in Australian coastal waters (Shaw *et al.* 2008).

The rates of N fixation for the Gulf do not take into account the role of N-fixing symbiotic cyanobacteria typically associated with diatoms, and small unicellular cyanobacteria which have recently been shown to contribute significantly to global N fixation rates (Carpenter *et al.* 1999; Zehr *et al.* 2001; Montoya *et al.* 2004; Capone *et al.* 2006). Previous studies in the Gulf have shown that diatoms with symbionts are numerically dominant, e.g. *Rhizosolenia* with *Richelia* (Burford *et al.* 1995). Therefore, N inputs from fixation are likely to be higher than the estimates calculated from this study.

Previous studies have shown that *Trichodesmium* is poorly grazed by zooplankton (Mulholland 2007). Studies in the Gulf have also shown that it is a poor nutritional source for shrimp larvae because the sheath surrounding the trichome is difficult to digest, resulting in the passing of intact trichomes through the gut. (Preston *et al.* 1998). However, *Trichodesmium* may contribute to N cycling via release of dissolved organic N and ammonium from cells (Glibert and Bronk 1994; Mulholland 2007) or viral lysis (Hewson *et al.* 2004). The released N has been shown to promote the growth of diatoms (Agawin *et al.* 2007). Alternatively the detrital material may be deposited on the sediment as it senesces. In our study, $\delta^{15}\text{N}$ ratios were lower in the sediment during a *Trichodesmium* bloom compared with non-bloom periods. Other studies have found isotopically light $\delta^{15}\text{N}$ ratios in sediment traps and sediment during *Trichodesmium* blooms (Capone *et al.* 1998; Knapp *et al.* 2005). Infaunal $\delta^{15}\text{N}$ ratios were not depleted during this time, suggesting that detrital material from the bloom was not substantially grazed by infauna and is therefore likely to be subject to microbial degradation.

During periods of water column stratification, particularly during summer months when wind mixing is low, nitrate can be higher in bottom waters (Rothlisberg *et al.* 1989; Rothlisberg *et al.* 1994; this study). This suggests that nitrification is occurring in or near the sediment. It is likely that deposition of N-rich cyanobacterial detritus generated from N fixation is an important source of N fuelling these bacterial processes. During winter months when the wind strength increases, the water column may become completely mixed and dissolved inorganic N from bottom waters becomes evenly distributed through the water column (Burford and Rothlisberg

1999). Therefore atmospheric N inputs via N-fixing cyanobacteria may create a coupling between water column and sediment processes, and hence also fuel primary productivity.

Water exchange

Net exchange of nutrients from the Arafura Sea is unlikely to be substantial given the similarity in the nutrient concentrations in the Gulf, Arafura Sea and the Torres Strait (Condie and Dunn 2006; M. Furnas, unpubl. data). Indeed, the deeper waters of the Gulf and the Arafura Sea have been classified within the same biogeographical region based on their physico-chemical and primary productivity characteristics (Condie and Dunn 2006). Additionally, water circulates around a central gyre in the Gulf meaning that the water residence time in the deeper waters of the Gulf is in the order of 3 y (Wolanski and Ridd 1990; Condie and Dunn 2006). The relatively low exchange of water in the northern Gulf, lack of difference between N concentrations entering and leaving the Gulf and low rates of denitrification mean that little N is likely to be lost from the system. Overall inputs of N were higher than outputs. It may be that N is accumulating or alternatively that N losses have been underestimated. A number of processes were not measured directly, such as anammox (anaerobic ammonium oxidation), which may have resulted in N removal.

The Gulf is a relatively pristine ecosystem, with the main nutrient inputs in the catchment likely to be from natural erosion processes, and low intensity agriculture (principally cattle grazing). Future agricultural development is proposed for the Gulf catchments including damming of rivers and irrigated intensive agriculture, e.g. ponded pastures and cotton production. Damming of rivers and regulation of flow may reduce nutrient loads hence reducing coastal production and negatively impacting on coastal fisheries. Conversely intensive forms of agriculture are likely to increase nutrient loads, in turn increasing estuarine and coastal production until a threshold is reached when ecosystem health declines as evidenced by anoxia and algal blooms.

In summary, this study has shown that productivity in the Gulf of Carpentaria is likely to be limited by N availability. In the deeper waters, the diazotroph *Trichodesmium* is likely to be an important source of new N as demonstrated diagrammatically (Fig. 6).

455 In shallow waters, N fixation and river inputs are likely to be relatively more
456 important. It is proposed that N fixed by *Trichodesmium* enters the food web via the
457 detrital pathway rather than directly by consumption by higher trophic levels.
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Figure Legend

Figure 1: Map showing study areas in the Gulf for the three voyages.

Figure 2: (a) Mean ($\pm$ SD) phosphate, nitrate, ammonia, and (b) silicate concentrations (μ M) in each region during voyage 3 in 2005.

Figure 3: $\delta^{15}\text{N}$ -nitrogen ratios ($\pm$ SD) in the particulate organic matter (open squares) and *Trichodesmium* (closed squares) in the water column during a *Trichodesmium* bloom in the Vanderlins, Groote Eylandt and Mornington Island regions in February/March 2005, and during a non-bloom period in the Vanderlins, Groote Eylandt, Mornington Island, Karumba and Albatross Bay offshore regions in January 2004 (closed diamonds) in the Gulf of Carpentaria

Figure 4: $\delta^{15}\text{N}$ -nitrogen ratios ($\pm$ SD) in the sediment on three separate sampling occasions in the Gulf of Carpentaria. Ka = Karumba region, Gr = Groote Eylandt region, We = Weipa offshore region, AB = Albatross Bay, Va = Vanderlins region, Mo = Mornington Island region.

Figure 5: Comparison of the mean $\delta^{15}\text{N}$ -nitrogen ratios ($\pm$ SD) of particulate organic matter and infauna in Albatross Bay, 4 sites, January 2005 (closed diamonds) and in Vanderlins (open circle), Groote Eylandt (open triangle) and Mornington Island (closed square) regions (3 sites in each region) February/March 2005 (open squares).

Figure 6: Schematic diagram showing the two zones in the Gulf: coastal waters and deeper waters, separated by a coastal boundary layer. The main direction of flow (black arrows) is shown in these two zones. Additionally, major sources of new N in the form of nitrogen fixation by plants (grey arrows) and river sources (white arrow) into these zones are shown.

Table 1: Summary of voyages to the Gulf of Carpentaria and parameters measured on each voyage. SIA = stable isotope analysis, POM = particulate organic matter, w/c = water column.

Voyage	Date	Region	Depth (m)	Parameters (# stations)
1	January 2004	Albatross Bay offshore	15-30	SIA POM (16) SIA sediment (16)
		Karumba offshore	10-25	
		Mornington Is.	35-40	
		Vanderlins	40-50	
		Groote Eylandt	35-40	
2	February 2005	Albatross Bay	7-10	SIA POM (4) SIA sediment (4) SIA infauna (4)
3	February – March 2005	Mornington Is.	35-40	SIA POM (10) SIA sediment (48) SIA infauna (9) Denitrification (29) N fixation w/c (1) Dissolved nutrients (123) Physico-chem profile (123) Chlorophyll <i>a</i> (9)
		Vanderlins	40-50	
		Groote Eylandt	35-40	

647 Table 2: Mean ($\pm$ SD) concentrations for physico-chemical parameters and nutrient
 648 rate measurements through the water column in February/March 2005 in the Gulf of
 649 Carpentaria. DIN = dissolved inorganic N.

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Parameter	Value (SD)	# Stations
Chlorophyll <i>a</i> ($\mu\text{g L}^{-1}$)	0.80 (0.45)	9
Oxygen (mg L^{-1})	5.98 (0.37)	123
Water temperature ($^{\circ}\text{C}$)	30.61 (0.36)	123
Denitrification rate ($\mu\text{mol m}^{-2} \text{h}^{-1}$)	0.27 (1.39)	29

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Table 3: Pools (MT N) and fluxes (MT N y⁻¹) of N in the Gulf of Carpentaria

Parameter	Pools (MT)	Fluxes MT y ⁻¹	Source
<i>Pools</i>			
Nitrate	75		Rothlisberg <i>et al.</i> (1989), Rothlisberg <i>et al.</i> (1994), Burford and Rothlisberg (1999), Burford <i>et al.</i> (this study)
Ammonium	24		Burford <i>et al.</i> (this study)
Regenerated inputs			
Nitrate uptake - phytoplankton		390	Burford and Rothlisberg (1999)
Ammonium uptake - phytoplankton		260	Burford and Rothlisberg (1999)
Sediment ammonium flux		?	
New inputs			
N fixation - <i>Trichodesmium</i>		290	Burford <i>et al.</i> (this study)
N fixation – seagrass beds		47	Moriarty and O'Donohue (1993), Pollard unpubl. data
N inputs - rivers		42	A. Brooks unpubl. Data, M. Burford unpubl. data
Outputs			
Denitrification		11	Burford <i>et al.</i> (this study)

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6