Sources of new nitrogen in the Indian Ocean

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Abstract Quantifying the different sources of nitrogen (N) within the N cycle is crucial to gain insights in oceanic phytoplankton production. To understand the controls of primary productivity and the associated capture of CO₂ through photosynthesis in the southeastern Indian Ocean, we compiled the physical and biogeochemical data from four voyages conducted in 2010, 2011, 2012, and 2013. Overall, higher NH₄⁺ assimilation rates (~530 μmol m⁻² h⁻¹) relative to NO₃⁻ assimilation rates (~375 μmol m⁻² h⁻¹) suggest that the assimilation dynamics of C are primarily regulated by microbial regeneration in our region. N₂ fixation rates did not decline when other source of dissolved inorganic nitrogen were available, although the assimilation of N₂ is a highly energetic process. Our data showed that the diazotrophic community assimilated ~2 nmol N L⁻¹ h⁻¹ at relative elevated NH₄⁺ assimilation rates ~12 nmol L⁻¹ h⁻¹ and NO₃⁻ assimilation rates ~6 nmol L⁻¹ h⁻¹. The small diffusive deep water NO₃⁻ fluxes could not support the measured NO₃⁻ assimilation rates and consequently point toward another source of dissolved inorganic NO₃⁻. Highest NO₃⁻ values coincided consistently with shallow lower dissolved O₂ layers (100–200 m; 100–180 μmol L⁻¹). These results suggest that nitrification above the pycnocline could be a significant component of the N cycle in the eastern Indian Ocean. In our analysis we provide a conceptual understanding of how NO₃⁻ in the photic zone could be derived from new N through N₂ fixation. We conclude with the hypothesis that N injected through N₂ fixation can be recycled within the photic zone as NH₄⁺ and sequentially oxidized to NO₂⁻ and NO₃⁻ in shallow lower dissolved oxygen layers.

1. Introduction

The supply of biologically available nitrogen (N) can be a bottleneck in the efficiency of the biological oceanic carbon pump. Nitrogen budgets in the open ocean regulate primary productivity and the associated fixation of C through photosynthesis [Ward et al., 2013]. The biological pump (sum of all the biologically mediated processes that export carbon) and the solubility pump (dissolution of CO₂ and its physical transport) are estimated to contribute equally to the CO₂ flux in the South Indian Ocean region [Valsala et al., 2012]. However, the regulation of the biological pump by the N cycle remains enigmatic despite the urgent need to understand productivity controls in the Indian Ocean [Alexander et al., 2012]. An understanding of potential alterations at the base of the food chain particularly reductions in planktonic biomass is essential, as a decline [Boyce et al., 2010] or a community shift [Montes-Hugo et al., 2009] in primary productivity will impact ecosystem services, such as O₂ production, carbon sequestration, biogeochemical cycling, and fisheries [Lehodey et al., 2010; Hollowed et al., 2013; Séférian et al., 2014].

The unique ability of diazotrophs to break the strong triple dinitrogen (N₂) bond (enthalpy = +945.5 kJ) makes them a potential long-term winner under climate change-driven reductions in inorganic N fluxes [Moore and Doney, 2007]. N₂ fixation by the diverse diazotrophic community has been shown to be a key regulator of the biological carbon pump [Moisander et al., 2010; Garcia et al., 2011]. Estimates show that N₂ fixation rates in the global oceans exceed 180 Tg N yr⁻¹ [Großkopf et al., 2012] and that N₂ fixation is able to support up to 50% of the new production in tropical low productivity areas such as the eastern Indian Ocean (IO) [Raes et al., 2014].

Dissolved inorganic nitrogen (DIN) concentrations in the photic zone of the eastern IO are often as low as 0.05 μmol L⁻¹ [Pearce and Pattiaratchi, 1999], with N:P ratios ~3 such that N strongly limits primary productivity in these oligotrophic waters [Hanson et al., 2007; Twomey et al., 2007]. Raes et al. [2014] have shown in this region that the highest DIN concentrations occur at low NO₃⁻:NH₄⁺ ratios. The latter suggest an active...
microbial community controlling mixed layer N and biogenic C fluxes through heterotrophic recycling, as seen in other systems via ammonification [Bouskill et al., 2012], nitrification [Yool et al., 2007], and N2 fixation [Karl et al., 2002].

In the northern parts of the eastern IO, Waite et al. [2013] have highlighted, via compound-specific isotopic measurements of the dissolved NO3− pool, that a high fraction of the NO3− (40−100%) in the photic zone can be derived from surface N2 fixation. For most of the oligotrophic waters in this oceanic basin the spatial scales of N2 fixation and its contribution to the dissolved NO3− pool plus the other sources of new and regenerated N remain uncharacterized components of the microbial N cycle.

In this manuscript we elucidate the key regional drivers of primary productivity in the southeastern IO using the physical and biogeochemical data sets from four regional voyages conducted during austral autumn and winter in 2010, 2011, 2012, and 2013. Physical mixing processes, dissolved inorganic nutrients, phytoplankton communities, and nutrient assimilation rates are assessed to investigate N cycling in this region. Our data suggest that part of the new N is delivered to the photic zone via N2 fixation rather than classic upwelling or mixing processes. We suggest that (a) N2 fixation products in the photic zone are recycled to NH4+ and that (b) these N2 fixation products are further nitrified (oxidized to NO2− and NO3−) in relatively lower dissolved oxygen (DO) layers within our study area. We propose that the classic separation between NO3− and NH4+ as the primary sources of new and recycled N [Dugdale and Goering, 1967; Eppley and Peterson, 1979] does not hold for these and possibly other similar regions of the world ocean.

2. Material and Methods

2.1. Study Region

We compiled data from four voyages aboard the R/V Southern Surveyor (SS) in the southeastern IO between 2010 and 2013 in the region bounded by 28°S−34°S and 110°E and 114°E in middle to late winter of each year (see station locations and time windows, Figure 1). Physical, biogeochemical data and metadata from 169 conductivity-temperature-depth (CTD) stations were accessed through the Integrated Marine Observing System (IMOS, http://www.imos.org.au). Temperature, salinity, DO, and photosynthetically active radiation (PAR) data were used to describe the physical oceanography, while dissolved inorganic nutrient concentrations including NO3−, NO2−, NH4+, Si, and PO4−3 were used to describe the regional chemical oceanography. Integrated chlorophyll a data were also compiled from the four different voyages. Dissolved inorganic nitrogen assimilation data were available from the 2011, 2012, and 2013 voyages. Pigment data and N2 fixation experiments were conducted during the 2012 and 2013 voyages.

2.2. Biophysical Data

Biophysical parameters were collected using a Seabird SBE911 conductivity-temperature-depth (CTD) profiler mounted on a rosette for all voyages. The profiler was fitted with a Seabird SBE32, 24-Niskin bottle rosette sampler, a Biospherical PAR sensor, a SBE43 O2 sensor, a Chelsea Aqua tracker Fluorometer, and a Wetlabs C-Star transmissometer, with measurements used as a proxy for particle concentration [Karageorgis et al., 2008]. Winkler titrations [Winkler, 1888] from each individual voyage were used to calibrate the SBE43 O2 sensor. Linear regression between O2 sensor and titration data for the respective voyages were \( r^2 = 0.9705 \) for SS2010v05, \( r^2 = 0.9607 \) for SS2011v04, \( r^2 = 0.9767 \) for SS2012v04, and \( r^2 = 0.9918 \) for SS2013v04. Sea surface heights were derived from satellite altimetry provided via IMOS (http://oceancurrent.imos.org.au/sourcedata/).

2.3. Nutrient Data

Dissolved inorganic nutrients for the SS2010v05 and SS2011v04 voyages were analyzed with a Lachat Autoanalyzer and with a Bran+luebbe AA3 HR segmented flow analyzer during the SS2012v04 and SS2013v04 voyages. Nutrients were analyzed aboard the ship following standard spectrophotometric methods [Hansen and Koroleff, 2009]. NO3−/NO2− was analyzed according to Armstrong et al. [1967] and Grasshoff et al. [2009] with detection limits to 0.015 μmol L−1. Phosphate was analyzed according to Murphy and Riley [1962] with detection limits to 0.01 μmol L−1. Silicate was determined according to Grasshoff et al. [2009] with detection limits to 0.01 μmol L−1. NH4+ concentrations were obtained according to Kérouel and Aminot [1997], later adapted by Watson et al. [2004] with detection limits to 0.004 μmol L−1. All calibration curves
had an $r^2$ of 0.999. Coefficients of variation were 0.2% for NO$_3^-$ and NO$_2^-$, 0.4% for PO$_4^{3-}$, 0.2% for NH$_4^+$, and 0.5% for Si. Long-term nutrient data (1951–2014) from the national reference station located near Rottnest Island (32.002°S; 114.416°E; Black circle on map in Figure 1b). All concentrations are in μmol L$^{-1}$. Silicate (Figure 1c) red line second-degree polynomial fit ANOVA $p < 0.0001$, NO$_3^-$ (Figure 1d) red line denotes fourth-degree polynomial fit ANOVA $p < 0.0001$; NH$_4^+$ concentrations (Figure 1e) were only available from 2010 to 2014, red line denotes fourth-degree polynomial fit ANOVA $p < 0.0001$; and PO$_4^{3-}$ (Figure 1f), red line fourth-degree polynomial fit ANOVA $p < 0.01$. Grey bars indicate when the voyages were conducted.

Voyage-specific high-resolution NO$_3^-$ data were derived from a third polynomial correlation between temperature and in situ NO$_3^-$ bottle data [King and Devol, 1979; Kamykowski et al., 2002]. Water mass-specific $r^2$ for the polynomials for the Leeuwin Current (LC) waters were $r^2 = 0.755, 0.964, 0.567, and 0.637$ in 2010, 2011, 2012, and 2013, respectively. For the Subtropical waters (STWs), we found $r^2 = 0.967, 0.982, 0.9664,
and 0.815 in 2010, 2011, 2012, and 2013, respectively. Mixed layer depths (MLDs) were calculated according to de Boyer Montégut et al. [2004] as a ΔT decrease of 0.4°C compared to a reference value at 6 m depth. As a comparison MLDs were also calculated as the depth where the potential density increased with 0.125 kg m⁻³ to a reference value at 6 m. These MLDs were rejected as they were inconsistent compared to the thermoclines from various CTD casts. We note that for all voyages all CTD casts were deeper than the MLD which is important to note as Greenwood and Craig [2014] reported that the MLD often extends to the seabed in our study region. Two widely used diffusive coefficients (Kz, s) (0.1 and 0.37 cm² s⁻¹) for oligotrophic waters were used to estimate a first-order range of the injection of new N over the mixed layer depth to provide a broader context of the supply of new N (Lewis et al. [1986] and Oschlies [2002], respectively). Flux calculations (\( \frac{\text{dNO}_3^-}{\text{d} z} = K_z s \text{dNO}_3^- / \text{d} z \)) were performed for the depth interval from 25 m below the MLD to 5 m above the MLD.

### 2.4. Biological Data

Chlorophyll a extractions were carried out according to Parsons et al. [1984] on 1 L water samples, using 25 mm Glass Microfiber Filter filters through gentle vacuum filtration (pressure drop < 10 kPa) at six sampling depths (up to 100 m). Samples were measured on a Turner design 10 AU fluorometer (for voyages SS2010v05 and SS2011v04) and on a Turner Trilogy fluorometer (for voyages SS2012v05 and SS2013v04). Pigment-specific analysis was carried out on 4 L water samples, using 25 mm Whatman GF/F filters through gentle vacuum filtration (pressure drop < 10 kPa). Pigments were analyzed to determine the phytoplankton communities using high-performance liquid chromatography (HPLC) according to the Commonwealth Scientific and Industrial Research Organisation (CSIRO) methods, see chapter 2 in Hooker et al. [2012]. Processed HPLC data were analyzed using the diagnostic pigments of the dominant phytoplankton taxonomic groups and are described in detail by Vidussi et al. [2001], Hirata et al. [2008], and Aiken et al. [2009]. All pigment data were quality controlled according to Aiken et al. [2009]. Our data showed that (1) the total Chl a (chlorophyll a; TChl a) made up at least 70% of the total pigment concentration and (2) the regression between TChl a and the accessory pigments had a slope of 1.1 and \( r^2 > 0.9 \). The microplankton community was defined as the sum of the diatom proportion (Fucoxanthin) and the dinoflagellate proportion (Peridinin) over the diagnostic pigments (DPs). The Nanoflagellate community was defined by the Alloxanthin +19'-Butanoyloxyfucoxanthin + Chlorophyll β + 19’-Hexanoyloxyfucoxanthin/DP ratio and the picoplankton community by the Zeaxanthin/DP ratio (see supporting information Table S1 for phytoplankton pigment abbreviations).

### 2.5. Assimilation Rates

Stable isotope tracers (\(^{15}\text{N}\)) were used to measure DIN assimilation rates during the 2011, 2012, and 2013 voyages. Dual labeling experiments with NaH\(^{15}\text{CO}_3\) and \(^{15}\text{N}\) tracers were conducted during the 2011 and 2013 voyages. During these voyages 20 \( \mu \text{mol L}^{-1} \) of NaH\(^{15}\text{CO}_3\) was added simulaneously to incubation bottles that were inoculated with \(^{15}\text{N}\) tracers including \(^{15}\text{NO}_3^-\), \(^{15}\text{NH}_4\text{Cl}\), and \(^{15}\text{N}_2\) [Waite et al., 2007a]. Water samples were taken at the surface (SFC), the deep chlorophyll maximum and at two additional depths for the 2011 voyage and at the surface (SFC), 20 m and 50 m below the SFC. \( K_z \) was calculated as the depth where the potential density increased with 0.125 kg m⁻³. Flux calculations (\( \frac{\text{dNO}_3^-}{\text{d} z} = K_z s \text{dNO}_3^- / \text{d} z \)) were calculated for the depth interval from 25 m below the MLD to 5 m above the MLD. The inoculated water samples were incubated for 6 h (from 8:00 h – 14:00 h) during the 2011 voyage and for 24 h during the 2012 and 2013 voyages. Regardless of incubation time all assimilation rates were in line with earlier recorded measurements [Hanson et al., 2007; Waite et al., 2007a]. Polycarbonate bottles were placed in on-deck incubators where temperature regulation was maintained by a continuous surface seawater flow. A range of neutral density screens were used to mimic light attenuation at different depths.

Limitations during the study were that \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) concentrations were unknown prior to the incubation experiments. We therefore based our spike concentrations on the trace additions of Waite et al. [2007a], Twomey et al. [2007], and Hanson et al. [2007]. Trace additions exceeding 10% did not enhance assimilation rates in our study area (\( r^2 = 0.003 \), slope = –0.0018, \( p = 0.57, n = 120 \) for \( \text{NO}_3^- \) assimilation rates and \( r^2 = 0.073 \), slope = –0.0106, \( p = 0.0019, n = 129 \) for \( \text{NH}_4^+ \) assimilation rates, respectively; see supporting information Figure S1).
$N_2$ fixation experiments during the 2012 voyage were conducted simultaneously using the methods described in Montoya et al. [1996] and according to Mohr et al. [2010], where the direct addition of $^{15}N_2$ tracer-enriched seawater was used as an alternative method to quantify $N_2$ fixation rates [White, 2012]. For the Montoya et al. [1996] methodology 5 mL $^{15}N_2$ gas at atmospheric pressure was added to the 4.5 L polycarbonate incubation bottles. For the Mohr et al. [2010], degassed and filtered (Sterivex filter 0.2 μm) YBC-II media was spiked with 1 mL $^{15}N_2$ gas at 90 atom%; Aldrich) gas per 100 mL and incubations were initiated by introducing aliquots of $^{15}N_2$ tracer-enriched seawater of 2.6% of the total incubation volume (4.5 L polycarbonate bottle) [see Raes et al., 2014]. During all experiments, the incubation bottles were gently rocked ~50 times to enhance mixing prior to 24 h incubation. The solubility of the $N_2$ concentration in seawater was calculated according to Hamme and Emerson [2004]. The recent discovery of potential contamination of $^{15}N_2$ gas with other forms of $^{15}N$ [Dabundo et al. 2014] cannot be ruled out; but if present, these contaminants had a negligible effect as our results clearly show a significant difference between theuptake of $N_2$, $NO_3^-$, and $NH_4^+$ (Figure 5). We also measured different $N_2$ fixation rates throughout the water column and between stations (contamination would give equal rates throughout the water column). We note that we used the same $^{15}N_2$ batch for a latitudinal transect presented in Raes et al. [2014]. Along this latitudinal transect all biotic and abiotic parameters as well as $N_2$ fixation rates correlated with latitude. Again, contamination would have skewed $N_2$ fixation rates. Lot number for the used $^{15}N_2$ gas canister was $SZ1670V$.

Here we report that the regression slope between the bubble and the dissolution method (slope = 0.51, $r^2 = 0.72$, $n = 30$, and $p < 0.0001$) from our measurements was very similar to the slope reported by Großkopf et al. [2012] (slope = 0.59, $r^2 = 0.6$). Our data compliment the discussion that $N_2$ fixation rates are underestimated when using the bubble method as highlighted by Mohr et al. [2010] and White [2012]. The $N_2$ fixation rates that are presented below are only derived from bioassays using the dissolution method.

All assimilation and fixation experiments were terminated by filtering each bottle (pressure drop < 10 kPa) through a 25 mm precombusted GF/F filter. Natural abundance samples of particulate organic carbon/particulate organic nitrogen, used as t zero values to calculate assimilation rates, were obtained by filtering 4 L water samples onto precombusted GF/F filters. Filters were snap frozen in liquid N and stored at −80°C. Filters were later acidified and dried overnight at 60°C. Determination of total C, total N, $δ^{13}C$, and $δ^{15}N$ were carried out using a continuous flow system consisting of a SERCON 20-22 mass spectrometer connected with an Automated N and C Analyzer. Multipoint normalization was used in order to reduce raw values to the international scale [Paul et al., 2007]. Error propagation for stable isotope data was performed as described by Skrzypek et al. [2010]. The external error of analyses (1 standard deviation) was 0.15‰ for $δ^{13}C$ and 0.20‰ for $δ^{15}N$. Nitrogen assimilation and C fixation rates ($\rho$ in mmol N or C L$^{-1}$ h$^{-1}$) were calculated following Dugdale and Goering [1967] and Knap et al. [1996].

### 2.6. Data Analysis

In situ oxygen, temperature, salinity, fluorescence, and PAR data within the mixed layer for each CTD station were analyzed using principal coordinate analysis (PCA) to separate the physical properties of the different water masses for each voyage. Nutrient concentrations for each CTD station were averaged over the MLD, samples size for averages are given in the text. Trapezoidally depth integrated Chl a values were derived from six sampling depths per CTD station down to 100 m for all voyages. Trapezoidally depth integrated $NO_3^-$, $NH_4^+$, and C assimilation rates from the SS2011v04 voyage were derived from four sampling depths per CTD station down to 100 m. Statistical analyses including PCAs, two- and one-way analysis of variance (ANOVA) tests were performed using the statistical package R v3.0.1 [R Development Core Team, 2013].

### 3. Results

#### 3.1. Physical Oceanography

We grouped our CTD stations into two water masses based on temperature (T), salinity (S), dissolved O$_2$ (DO), fluorescence, and photosynthetic active radiation (PAR). Scatterplots, based on the principal coordinate (PC) loadings, visualized the clustering of the different stations into Subtropical water (STW n = 79 CTD stations) and Leeuwin Current water (LC n = 98 CTD stations) for all voyages (Figure S2). Temperature, salinity, and dissolved O$_2$ concentrations explained more than 50% of the variance (first PCs; Figure S2 and Table 1).
Table 1. Eastern IO

<table>
<thead>
<tr>
<th>Year</th>
<th>LC</th>
<th>STW</th>
<th>LC</th>
<th>STW</th>
<th>LC</th>
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<tbody>
<tr>
<td>2010</td>
<td>21.2 ± 0.5</td>
<td>18.7 ± 0.5</td>
<td>20.1 ± 0.5</td>
<td>19.1 ± 0.8</td>
<td>21.7 ± 0.7</td>
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<td>2011</td>
<td>23.8 ± 0.4</td>
<td>21.4 ± 0.4</td>
<td>24.1 ± 0.4</td>
<td>23.3 ± 0.3</td>
<td>25.3 ± 0.5</td>
<td>24.2 ± 0.5</td>
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<td>2012</td>
<td>23.5 ± 0.3</td>
<td>21.0 ± 0.3</td>
<td>24.1 ± 0.4</td>
<td>23.5 ± 0.3</td>
<td>25.4 ± 0.5</td>
<td>24.8 ± 0.5</td>
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<tr>
<td>2013</td>
<td>23.3 ± 0.2</td>
<td>21.2 ± 0.2</td>
<td>24.1 ± 0.3</td>
<td>23.4 ± 0.2</td>
<td>25.3 ± 0.4</td>
<td>24.7 ± 0.4</td>
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**To MLD** (m) | LC | STW | LC | STW | LC | STW |
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<tr>
<td>2010</td>
<td>212 ± 0.5</td>
<td>187 ± 0.5</td>
<td>201 ± 0.5</td>
<td>191 ± 0.8</td>
<td>217 ± 0.7</td>
<td>207 ± 0.7</td>
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<tr>
<td>2011</td>
<td>234 ± 0.4</td>
<td>214 ± 0.4</td>
<td>244 ± 0.4</td>
<td>233 ± 0.3</td>
<td>254 ± 0.5</td>
<td>242 ± 0.5</td>
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<tr>
<td>2012</td>
<td>233 ± 0.3</td>
<td>210 ± 0.3</td>
<td>241 ± 0.4</td>
<td>235 ± 0.3</td>
<td>254 ± 0.5</td>
<td>248 ± 0.5</td>
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<tr>
<td>2013</td>
<td>233 ± 0.2</td>
<td>212 ± 0.2</td>
<td>241 ± 0.3</td>
<td>234 ± 0.2</td>
<td>253 ± 0.4</td>
<td>247 ± 0.4</td>
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**Temperature (°C)** | LC | STW | LC | STW | LC | STW |
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<tr>
<td>2010</td>
<td>21.2 ± 0.5</td>
<td>18.7 ± 0.5</td>
<td>20.1 ± 0.5</td>
<td>19.1 ± 0.8</td>
<td>21.7 ± 0.7</td>
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<tr>
<td>2011</td>
<td>23.8 ± 0.4</td>
<td>21.4 ± 0.4</td>
<td>24.1 ± 0.4</td>
<td>23.3 ± 0.3</td>
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<td>24.2 ± 0.5</td>
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<tr>
<td>2012</td>
<td>23.5 ± 0.3</td>
<td>21.0 ± 0.3</td>
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<tr>
<td>2013</td>
<td>23.3 ± 0.2</td>
<td>21.2 ± 0.2</td>
<td>24.1 ± 0.3</td>
<td>23.4 ± 0.2</td>
<td>25.3 ± 0.4</td>
<td>24.7 ± 0.4</td>
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**Yearly comparison between LC and STW water masses are in bold (p < 0.01); data are average ± standard deviation (SD); n = 45 CTD stations for 2010, n = 38 CTD stations for 2011, n = 31 CTD stations for 2012, and n = 38 CTD stations for 2013.**

The mean mixed layer depths in the LC (100 ± 54 m ± standard deviation (SD)) and in the STW (92 ± 29 m ± SD) were not significantly different (one-way ANOVA p = 0.615). Dissolved O₂ concentrations were always significantly lower in the poleward flowing LC than in the STW (Table 1). Layers of relatively lower dissolved O₂ [Thompson et al., 2011] were observed between 100 and 250 m within and below the mixed layer depth during all voyages (see supporting information Figure S3c) and were associated with warm, low-density, and low-salinity waters. Localized reductions in transparency, an indication of increased particle concentrations, were associated with LC waters (data not shown). Local hot spots of O₂ depletion also occurred within warm core eddies (~180–220 μmol L⁻¹; 100–250 m; see Figure S4).

### 3.2. Chemical Oceanography

Across the 4 years, mean NO₃⁻ concentrations within the mixed layer in the LC were 0.2 μmol L⁻¹ (n = 349) and were significantly higher than average NO₃⁻ concentrations in the STW (0.08 μmol L⁻¹, n = 303; Table 1). Nitrate concentrations were elevated (up to 0.3 μmol L⁻¹) at reduced O₂ concentrations ~180–200 μmol L⁻¹ between 100–250 m at the base of the LC. Warmer LC waters had lower O₂ and higher NO₃⁻ concentrations than the STW (Table 1). The shape of vertical profiles of nutrients, especially in deep mixed layers (MLD ~200 m), will have a significant bearing on the calculation of the vertical NO₃⁻ flux (see below). The vertical profile of NO₃⁻ within the LC showed a small but significant increase with increasing MLD, (slope = 0.0016 μmol L⁻¹ m⁻¹; Figure S5a). In the STW distribution of NO₃⁻ was uniform within the mixed layer (Figure S6a).

Average NH₄⁺ concentrations were 5 times lower than the average NO₃⁻ concentrations in the surface mixed layer. NH₄⁺ concentrations were significantly greater in the LC than the STW in 2010 only (Table 1). Across all years, NH₄⁺ concentrations were marginally higher in the LC waters than the STW (one-way ANOVA p = 0.06). The vertical profile of NH₄⁺ was uniform in both water masses (Figures S5b and S6b).

The LC carried greater mixed layer PO₄³⁻ concentrations than the STW (Table 1), with a small yet significant increase with depth (slope = 0.002; Figure S5c). In the STW waters PO₄³⁻ concentrations were uniform within the MLD (Figure S6c). NO₃⁻:PO₄³⁻ ratios in the LC were higher than in the STW (one-way ANOVA p < 0.001; in the LC, NO₃⁻:PO₄³⁻ = 2 ± 2.2 (±SD, n = 347) versus 1 ± 1.6 (±SD, n = 296) in the STW.) These NO₃⁻:PO₄³⁻ ratios are ~5 to 10% of global ratios [e.g., Redfield, 1958] and highlight the oligotrophic and N-limited nature of this ecosystem.

The eastern Indian Ocean is low in DIN and phosphate but showed high concentrations of silicate (up to 4.7 μmol L⁻¹). The LC plays a role as a silicate source [Lourey et al., 2006].
The oligotrophic nature of the eastern Indian Ocean relates to an overall low (~0.35 μg L⁻¹) standing stock (Table 1). Although the average Chl a concentrations were significantly greater in the LC than in the STW during the 2010 and 2012 voyages (Table 1), no significant differences were found for the depth-integrated Chl a concentrations between the two water masses across all other years (one-way ANOVA p > 0.2 for all voyages). We therefore combined the LC and STW stations to get a regional picture of new N in the euphotic zone is ~3 times lower in LC waters, highlighting the upwelling suppressing nature of the poleward flowing Leeuwin Current.

3.3. Seasonal Changes

To nest this analysis in a broader temporal picture of the oligotrophic nature of our region, we analyzed nutrient data over a 60 year period. The data, collected from five depths (0–50 m) from the national reference station at Rottnest Island in the southern quadrant of our research area, highlight the seasonal trend of nutrient concentrations in the eastern IO (Figures 1c–1f). We note that despite significant seasonal fluctuations, the average DIN and PO₄³⁻ concentrations always remained < 1 μmol L⁻¹. NO₃⁻ concentrations showed a significant increase (up to 0.8 μmol L⁻¹) in June, whereas NH₄⁺ concentrations displayed a peak between April and June around 0.3 μmol L⁻¹. Phosphate showed the lowest degree of temporal variability, with concentrations showing a minimal increase around June. Of all nutrients, silicate concentrations had the clearest temporal variability, increasing from April through September (Figures 1c–1f).

3.4. Biological Oceanography

3.4.1. Pigments

The oligotrophic nature of the eastern Indian Ocean relates to an overall low (~0.35 μg L⁻¹) standing stock (Table 1). Although the average Chl a concentrations were significantly greater in the LC than in the STW during the 2010 and 2012 voyages (Table 1), no significant differences were found for the depth-integrated Chl a concentrations between the two water masses across all other years (one-way ANOVA p > 0.2 for all voyages). We therefore combined the LC and STW stations to get a regional picture of Chl a concentrations in the eastern IO. Depth-integrated Chl a concentrations from four austral voyages averaged 42 ± 15 mg m⁻² and showed a positive correlation with sea surface height across all years (Figure 2).

3.4.2. Communities

Regionally, the nanoplancton represented the bulk (~60%) of the functional phytoplankton communities. Nanoplankton was the most abundant with a mean fraction of 0.59 ± 0.17, where after pico plankton with
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There was no significant difference in the size structures in the LC or the STW. One-way ANOVA, p = 0.843, p = 0.584, and p = 0.562 for the micro, nano, and pico size classes in the LC, respectively; and one-way ANOVA, p = 0.078, p = 0.812, and p = 0.969 for the micro, nano, and pico size classes in the STW, respectively.

3.4.3. NH₄⁺ and NO₃⁻ Assimilation Rates

NH₄⁺ and NO₃⁻ assimilation rates were not significantly different between the two water masses in 2011 or 2012, and rates from the two water masses were therefore pooled to get a regional picture of DIN incorporation rates. Regional depth-integrated NH₄⁺ assimilation rates in 2011 (532 ± 217 μmol m⁻² h⁻¹) were significantly higher than NO₃⁻ assimilation rates in 2013, NH₄⁺ assimilation rates were higher than NO₃⁻ assimilation rates in LC waters than in the STW (one-way ANOVA p < 0.01). No significant differences, however, were found for the NO₃⁻ assimilation rates in the LC and STW (one-way ANOVA p = 0.345).

From a regional perspective NH₄⁺ assimilation rates were 68% greater relative to the NO₃⁻ assimilation rates (one-way ANOVA p < 0.001; Table 3). Across three cruises in 2011, 2012, and 2013 the maximum assimilation of NH₄⁺ was consistently greater, and in some years up to 2.7 times greater, compared to the NO₃⁻ assimilation rates (Table 3), there was no significant difference between the NH₄⁺ assimilation means across the years (one-way ANOVA p = 0.155).

3.4.4. N₂ and C Fixation

Regionally, during the 2012 voyage, N₂ fixation rates ranged from 0.1 to 12.6 nmol L⁻¹ h⁻¹ (Table 3). N₂ fixation rates were much lower in the relatively lower oxygenated layers (~180 μmol L⁻¹) compared to well-oxygenated surface waters (Figure 4). During our 2013 voyage regional N₂ fixation rates were significantly smaller from those found in the previous year (one-way ANOVA p < 0.01 Table 3). In both years N₂ fixation rates in the LC were significantly greater compared to the rates in the STW (one-way ANOVA’s p < 0.01; Figure 4). We did not depth integrate our N₂ fixation rates as we only had two vertical measurements; one at the surface (6 m) and one in the lower oxygenated waters (~100–200 m).

Carbon fixation rates averaged 21.7 ± 6.11 nmol L⁻¹ h⁻¹ (SD, n = 102) in 2011 and 21.3 ± 16.16 nmol L⁻¹ h⁻¹ (SD, n = 32) in 2012, and were not significantly different between the LC and the STW (one-way ANOVA p = 0.254). Dual labeling experiments (¹⁵N and¹³C), during the 2013 voyage, showed that all DIN assimilation rates correlated positively with C fixation rates.

Figure 2. Depth-integrated Chl a concentrations (mg m⁻²) plotted against sea surface height (m). Red line presents linear regression r² = 0.11, p < 0.0001, slope = 38, n = 149.

Figure 3. Phytoplankton community composition in the eastern IO. Size classes picophytoplankton (Pico), nanophytoplankton (Nano) and microphytoplankton (Micro) for the SS2012v04 and SS2013v04 voyages. Internally, the phytoplankton community composition did not show a significant difference in their size structures in the LC or the STW. One-way ANOVA, p = 0.843, p = 0.584, and p = 0.562 for the micro, nano, and pico size classes in the LC, respectively; and one-way ANOVA, p = 0.078, p = 0.812, and p = 0.969 for the micro, nano, and pico size classes in the STW, respectively.
The slope of the DIN assimilation and C fixation rates converted to an apparent C:N ratio of 6 for NH$_4^+$ assimilation, an apparent C:N ratio of 11.7 for NO$_3^-$/C$_0$ assimilation, and an apparent C:N ratio of 47.8 for N$_2$ assimilation (Figure 5). However, basing the C:N ratios on single DIN assimilation rates (e.g., C versus NH$_4^+$ only) could overestimate the true C:N assimilation ratio. The combined $^{13}$C: ($^{15}$NO$_3^-$ + $^{15}$NH$_4^+$ + $^{15}$N$_2$) ratio, regionally, averaged 4 ± 2.2 (±SD, $n$ = 32).

4. Discussion

4.1. N Cycling in the Indian Ocean

In our study, the water mass separation between the Leeuwin Current (LC) and Subtropical Water Mass (STW) did not necessarily translate into significantly different biological responses such as phytoplankton size classes, biomass, and C and N assimilation rates. We therefore constructed a more regional oceanographic picture of how the N cycle influences primary productivity, nesting this in long-term nutrient data from the Rottnest Island (IMOS http://www.imos.org.au/). We then calculated the diapycnal NO$_3^-$/C$_0$ fluxes and the DIN assimilation rates over the 4 year period of our study, to estimate the overall contribution of N$_2$ fixation to new N.

The Indian Ocean (IO) is potentially one of the most N-limited regions in the world ocean [Polovina et al., 2008]. The average seasonal distribution of DIN stays below 1 $\mu$mol L$^{-1}$, and N:P ratios average around 12–13 in our study, which are similar to those documented in the World Ocean Circulation Experiment (WOCE) Atlas for the IO [Talley, 2013]. Nutrient ratios presented herein from the mixed layer were even lower than the WOCE values, suggesting a preferential net drawdown of N with respect to P. The N-limited nature of this ecosystem is also highlighted in the seasonal data by the excess of PO$_4^{3-}$ and the overabundance of Si in comparison to DIN. The lack of a clear linear relationship between P and N in the mixed layer, and the high level of scatter in our seasonal DIN data at low concentrations, made it difficult to resolve the regional impact of N$_2$ either from actual nutrient concentrations or from stoichiometric concepts such N$^*$ and P$^*$ alone [see Deutsch et al., 2001].

Our efforts to calculate a regional N$^*$ value did not give any insights despite the fact that N approached the detection limit which suggests the potential for significant N injections through N$_2$ fixation.
The relatively low volumetric and depth-integrated NO$_3^-$ assimilation rates (≈15 nmol L$^{-1}$ h$^{-1}$; ~375 μmol m$^{-2}$ h$^{-1}$) were in line with earlier measurements in this region [Hanson et al., 2007; Waite et al., 2007a]. The estimated vertical diffusive NO$_3^-$ fluxes, however, over the mixed layer depth were 10 times lower than the reported fluxes from the open Atlantic (~140 μmol m$^{-2}$ d$^{-1}$ [Lewis et al., 1986], but of similar order to the fluxes reported by Planas et al. [1999] in the North Atlantic subtropical gyre ~50 μmol m$^{-2}$ d$^{-1}$). Depth-integrated NO$_3^-$ assimilation rates thus exceeded the diffusive flux (~1–30 μmol m$^{-2}$ d$^{-1}$; Table 2) by a factor of 100. Planas et al. [1999] noted a strong nonlinear relationship between NO$_3^-$ assimilation rates and the deep NO$_3^-$ supply. They reported that NO$_3^-$ assimilation rates exceeded the deep NO$_3^-$ flux by a factor of 5 in the oligotrophic central Atlantic. Elevated assimilation rates along with the presence of low NO$_3^-$ concentrations are typical in recently upwelled waters nearing the end response of a phytoplankton bloom [Dugdale et al., 1990]. The latter scenario is, however, not relevant to the southeastern IO as the poleward flowing LC suppresses upwelling [Waite et al., 2007b] and NO$_3^-$ concentrations are typically relatively low (see seasonal data). Although NO$_3^-$ assimilation was consistently a small fraction of the total N assimilation, it is clear that a simple vertical diffusive flux still does not suffice to supply the measured NO$_3^-$ demand. Therefore, an alternative NO$_3^-$ source must be identified to complete the N mass balance for the region.

### 4.3. New N From Below the MLD

In our study the overall 68% higher NH$_4^+$ assimilation rates relative to NO$_3^-$ assimilation rates suggest that the fixation dynamics of dissolved inorganic carbon are regulated primarily by microbial regeneration of particulate organic matter and dissolved organic matter (DOM) [Azam et al., 1994; Azam, 1998; Verdugo et al., 2004].

OTOLITHIC pelagic regions are mostly characterized by the microbial food web where protozoans graze on the picophytoplankton and nanophytoplankton [Cushing, 1989]. Low nutrient concentrations favor smaller phytoplankton size classes with a higher affinity for regenerated N [Dorothy, 1990; Epopey et al., 1969]. This correlation is confirmed by our HPLC data as the nanophytoplankton represented >60% of the phytoplankton community. The higher nutrient affinity of the smaller size fractions in these nutrient-depleted waters, nearly all year round provides a potential explanation for the (relative) paucity of diatoms despite seasonal oversupply of Si.

### 4.4. Other Potential N Sources

Warm core eddies (positive sea surface heights) are a common feature of the Leeuwin Current [Feng et al., 2007] and have been shown to exhibit locally enhanced primary productivity via a variety of mechanisms, including cross-shelf nutrient enrichment [Waite et al., 2015], frictional decay [Franks et al., 1986], retention of nutrients [Greenwood et al., 2007], injection of new N through N$_2$ fixation [Fong et al., 2008], seasonal deepening of the mixed layer due to heat loss and pycnocline deflection [Pearce and Feng, 2007]. Other sources of NO$_3^-$ could be the vertical advection of nutrients via submesoscale upwelling which has been documented by Paterson et al. [2008] in our study area. We note that on a seasonal scale (especially in winter), the importance of mixed layer nutrient entrainment with a deepening of the MLD, as highlighted by Dufais et al. [2014], will be an
important residual source of NO$_3^-$ . The importance of N injections through these events is highlighted by the significantly higher integrated Chl a concentrations associated with positive sea surface heights across the study region as seen by Feng et al. [2003] and Waite et al. [2007b]. These Chl a anomalies with positive sea surface heights have been observed to extend beyond the LC region across the whole southern IO [Brewin et al., 2012; Dufois et al., 2014].

Global modeling studies have also highlighted the role of subscale and mesoscale turbulence in providing additional N sources in the euphotic zone [McGillicuddy and Robinson, 1998]. Horizontal nutrient transport and nutrient streams [Williams et al., 2006] can enhance new production [Williams and Follows, 1998] including the lateral transport of dissolved organic nitrogen (DON) [Torres-Valdés et al., 2009], When such episodic nutrient injections occur, the contribution of N$_2$ fixation will have a lower importance. Oschlies and Garçon [1998] note that mesoscale turbulence can account for up to one third of the total NO$_3^-$ flux in the subtropics and midlatitudes of the North Atlantic Ocean. Yet they also state that this injection is not sufficient to maintain the observed primary production. Export production, by definition, requires a supply of new nutrients to the euphotic zone to offset sedimentary losses. Again, for the eastern Indian Ocean, sources of N other than cross-pycnocline transport must be considered.

### 4.5. N$_2$ Fixation

Besides the possible additional nutrient injections through the mesoscale processes (horizontal and lateral), we measured N$_2$ fixation rates at all stations during two consecutive years. Our N$_2$ fixation rates (<12 nmol L$^{-1}$ h$^{-1}$) fall between those presented by Montoya et al. [2004] in the Timor Sea (20–60 nmol L$^{-1}$ h$^{-1}$) and those reported by Großkopf et al. [2012] in the South Atlantic Ocean (~0.5 nmol L$^{-1}$ h$^{-1}$). One possible explanation for the relative high rates in the eastern IO compared to the South Atlantic Ocean could be the impact of dust transport, originating from Lake Eyre and from local sources such as the iron-rich Pilbara region [Morris and Ramananlou, 2007]. McGowan and Clark [2008] mapped a dust trajectory path that extends over a vast region covering the entire northern Australian coast from the Torres Strait (142°E, 10.5°S) to the Northwest Cape (114°E, 21°S). The core of the pathway passes over the Great Sandy Desert and the southern Kimberly into the IO and Timor Sea [McGowan and Clark, 2008], Dust deposition into nutrient-poor waters has been shown to stimulate marine productivity [Boyd et al., 2000; Jickells et al., 2005] and N$_2$ fixation rates [Mahaffey et al., 2004; Rubin et al., 2011].

Our N$_2$ fixation rates confirm model estimates [Monteiro et al., 2010; Monteiro et al., 2011] demonstrating that these rates in the southeastern IO are globally significant. The model from Monteiro et al. [2010] predicts a dominance of unicellular cyanobacterial N$_2$ fixation in the IO. These model data also support earlier findings from McClines et al. [2014] and Raes et al. [2014] where high bulk N$_2$ fixation rates (~6 nmol L$^{-1}$ h$^{-1}$) at higher latitudes (~35°S) were attributed to unicellular N$_2$ fixation. The N$_2$ fixation rates we have presented here further support the conclusion of Goebel et al. [2010] and Moisander et al. [2010] that in the eastern Indian Ocean, at least on a local-scale, unicellular N$_2$-fixing cyanobacteria can have equal or greater N$_2$ fixation rates than the cyanobacteria Trichodesmium.

Although the assimilation of N$_2$ is highly energetically demanding (enthalpy = +945.5 kJ), suggesting that fixation rates should decline when less expensive N sources are available [Weber and Deutsch, 2014], the fixation of N$_2$ did not decline when other sources of DIN were available in our study region. Our data showed that the diazotrophic community still fixed ~2 nmol L$^{-1}$ h$^{-1}$ even at relatively elevated NH$_4^+$ and NO$_3^-$ assimilation rates (~12 nmol L$^{-1}$ h$^{-1}$ and ~6 nmol L$^{-1}$ h$^{-1}$, respectively). These data suggest a continuous input of new N through N$_2$ fixation. With a first-order budget calculation we give a first estimate of how much N$_2$ fixation contributes toward N assimilation. N$_2$ fixation rates averaged 2.5 nmol L$^{-1}$ h$^{-1}$, NH$_4^+$ assimilation rates averaged 7 nmol L$^{-1}$ h$^{-1}$ and NO$_3^-$ assimilation rates averaged 2.6 nmol L$^{-1}$ h$^{-1}$ (see Table 3; 2012 and 2013 voyages). The total DIN assimilation rates averaged 12.1 nmol N L$^{-1}$ h$^{-1}$. The contribution of N$_2$ toward the total DIN assimilation pool averaged therefore 20% during the winter months in the eastern Indian Ocean, making fixation equal to NO$_3^-$ in terms of N assimilation. Our seasonal nutrient analysis shows that nutrients are elevated during winter months, leaving a potential for still higher N$_2$ fixation rates during summer. The sequestration of C by the diazotrophic community has been recognized as a core component in oceanic and atmospheric CO$_2$ coupling [Falkowski, 1997; Douglas, 2001], yet to date the quantification of N$_2$ fixation and the associated C sequestration still remains largely unknown, particularly in the IO.
4.6. Further Hypothesis

The entrainment of thin (10–20 m) and shallow (100–200 m) reduced oxygen layers at the base of the Leeuwin Current were detected by Rochford [1969] and described by Woo and Pottiwaratchi [2008]. Their association with biogeochemical anomalies including increased NO$_3^-$ concentrations [Thompson et al., 2011] and lowered pH [Waite et al., 2013] were identified later. Prior work in the northeastern IO links the oxidation of particulate organic matter associated with Low Dissolved Oxygen High Nitrate layers to the shallow regeneration of NO$_3^-$ sourced from newly fixed N within surface waters (NO$_3^-$ with a $^{15}$N signature ~0‰) [Waite et al., 2013]. From this and our current analysis we hypothesize that NO$_3^-$ can be sourced from the oxidation of the products of N$_2$ fixation, including particulate organic matter and NH$_4$+, in our study area. These concepts compliment the work of Ward et al. [1989] where rapid N recycling within the nitracline is highlighted along with the work of Mahaffey et al. [2004] where diazotrophs increased the DON pool in the northern oligotrophic subtropical gyre of the Atlantic Ocean. Mourino-Carballido et al. [2011] have furthermore shown that N$_2$ fixation can explain ~40% of the new N entering the euphotic zone in areas with low diffusive NO$_3^-$ fluxes and contribute up to half of the new production [Karl et al., 1997]. A NO$_3^-$ excess has further been linked to N$_2$ fixation in the North Atlantic Ocean by Hansell et al. [2004], where they modeled that the injection of NO$_3^-$ through nitrification of N-rich organic matter (derived from N$_2$ fixation) can be up to 13.5%. Painter et al. [2013] showed that the input of new N from N$_2$ fixation can be equivalent to up to 60% of the diffusive NO$_3^-$ supply and that N$_2$ fixation could exceed the diffusive flux at certain stations in the eastern subtropical North Atlantic Ocean. Taking these arguments into account, we suggest that the direct and potentially the indirect contributions of N$_2$ fixation could be an important source of new N in the eastern Indian Ocean. We therefore hypothesize that shallow nitrification could be a likely source for the relative high NO$_3^-$ assimilation rates we observed.

4.7. Conclusion

In this study we have provided evidence that N$_2$ fixation could be an important source of new N in the eastern Indian Ocean, supporting primary productivity. Although we lack nitrification rate measurements, our results are consistent with the theory of Thompson et al. [2011] which suggest that shallow nitrification is a significant component of the N cycle in the eastern IO. Our data complements the results of previous work of Waite et al. [2013] and Raes et al. [2014] and has advanced our understanding of new N recycling above the pycnocline in the southeastern Indian Ocean. The significance of nitrification above the pycnocline has been highlighted by other authors [Hansell et al., 2004; Yool et al., 2007; Ward et al., 2013; Ryabenko et al., 2012], yet global data on nitrification rates in surface waters are sparse. One consequence of surface nitrification is an overestimation of the new N attributed to NO$_3^-$ fluxes [Yool et al., 2007]. We therefore hypothesize that as the vertical flux of NO$_3^-$ from below the nutricline is not a dominant source of N that the bulk of the NO$_3^-$ could be derived from new N through N$_2$-fixation. Thus, new N injected through N$_2$-fixation could potentially be recycled within the photic zone to NH$_4^+$ and sequentially oxidized to NO$_3^-$ and NO$_2^-$. These amonified and oxidized forms of N, derived from N$_2$ fixation, could thereby potentially act as a new source of bioavailable N in the southeastern Indian Ocean.

Acknowledgments

We thank the captain and Marine National Facility crew of the Southern Surveyor for their technical assistance while at sea. Supporting grants for the SS2010v05 and SS2011v04 came from A.M. Waite. This research was made possible because Helen Phillips gave us the opportunity to piggyback onto the SS2013v04 and 2013v04 voyages. Eric J. Raes has been supported through an Australian Postgraduate scholarship from the University of Western Australia and a CSIRO Wealth from Oceans postgraduate top-up scholarship. We would like to thank Hannipoula Olsen for the experimental setup at sea. Physical, biogeochemical data and metadata to support this article can be accessed through the Integrated Marine Observing System (IMOS http://www.imos.org.au/). Biological assimilation data sets can be requested through the corresponding author. We sincerely thank the constructive feedback and insightful comments from the anonymous reviewers.

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