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# Influence of open ocean nitrogen supply on the skeletal $\delta^{15}\text{N}$ of modern shallow-water scleractinian corals



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## ABSTRACT

The isotopic composition of skeleton-bound organic nitrogen in shallow-water scleractinian corals (hereafter, CS- $\delta^{15}\text{N}$ ) is an emerging tool for studying the marine nitrogen cycle in the past. The CS- $\delta^{15}\text{N}$  has been shown to reflect the  $\delta^{15}\text{N}$  of nitrogen (N) sources to corals, with most applications to date focusing on the anthropogenic/terrestrial N inputs to reef environments. However, many coral reefs receive their primary N sources from the open ocean, and the CS- $\delta^{15}\text{N}$  of these corals may provide information on past changes in the open ocean regional and global N cycle. Using a recently developed persulfate/denitrifier-based method, we measured CS- $\delta^{15}\text{N}$  in modern shallow-water scleractinian corals from 8 sites proximal to the open ocean. At sites with low open ocean surface nitrate concentrations typical of the subtropics and tropics, measured CS- $\delta^{15}\text{N}$  variation on seasonal and annual timescales is most often less than 2‰. In contrast, a broad range in CS- $\delta^{15}\text{N}$  (of ~10‰) is measured across these sites, with a strong correlation between CS- $\delta^{15}\text{N}$  and the  $\delta^{15}\text{N}$  of the deep nitrate supply to the surface waters near the reefs. While CS- $\delta^{15}\text{N}$  can be affected by other N sources as well and can vary in response to local reef conditions as well as coral/symbiont physiological changes, this survey indicates that, when considering corals proximal to the open ocean, the  $\delta^{15}\text{N}$  of the subsurface nitrate supply to surface waters drives most of the CS- $\delta^{15}\text{N}$  variation across the global ocean. Thus, CS- $\delta^{15}\text{N}$  is a promising proxy for reconstructing the open ocean N cycle in the past.

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## 1. Introduction

Coral skeleton-bound organic matter (CSOM) constitutes 0.01–0.1% of the skeleton material by weight; and research is ongoing to understand the synthesis, composition and role of organic matter during the calcification process (Drake et al., 2013; Tambutte et al., 2011). From a paleoceanographic and biogeochemical perspective, the CSOM is directly synthesized by coral at the

time of calcification and may provide important information about coral reef environments in the past. For shallow-water scleractinian corals, CSOM has several key virtues as an archive of past conditions. First, CSOM is protected by the carbonate skeleton and may be preserved for tens or hundreds of millions of years (Muscatine et al., 2005). Second, shallow-water scleractinian corals are widely distributed in the low latitude ocean, and fossil coral samples are found throughout the Mesozoic and Cenozoic Eras (i.e. back to ~240 Ma). Third, shallow-water scleractinian corals have high linear extension rates (e.g., 2 cm/yr) and produce annual growth bands. Thus, appropriate techniques would allow for the generation of high-resolution records on individual coral cores.

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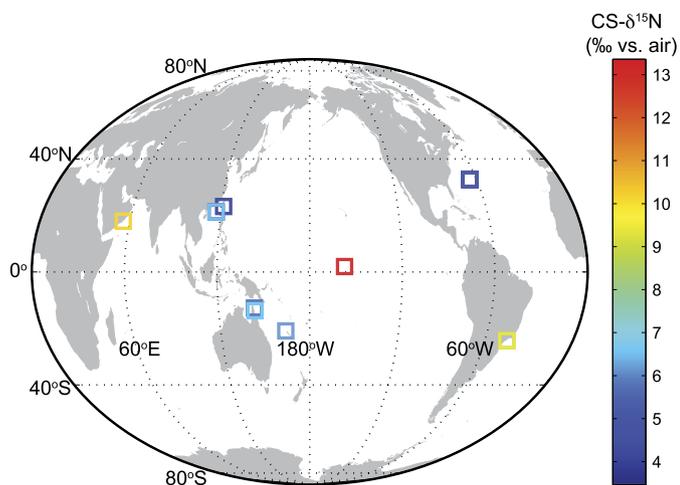


Fig. 1. Locations and average coral skeletal  $\delta^{15}\text{N}$  (CS- $\delta^{15}\text{N}$ , ‰ vs. air) for each coral core or set of cores used in this study.

Due to the difficulty associated with analyzing this dilute form of organic matter, only a handful of measurements have been made on CSOM: total organic carbon and amino acid composition (Ingalls et al., 2003), carbon isotopes (Muscatine et al., 2005) and nitrogen isotopes (Erler et al., 2015; Hoegh-Guldberg et al., 2004; Marion et al., 2005; Muscatine et al., 2005; Wang et al., 2015). Among these measurements, a recent analytical advance in nitrogen isotopic analysis of CSOM (hereafter: CS- $\delta^{15}\text{N}$ ) requires only 5–10 mg of carbonate material per measurement and yields a precision of 0.2‰ (Wang et al., 2015). Thus, this technique allows for the generation of seasonal or even monthly CS- $\delta^{15}\text{N}$  records on single coral cores that are comparable to other records made on the inorganic carbonate of corals (e.g.,  $\delta^{18}\text{O}$ , Metal/Ca ratio) (Erler et al., 2016).

CS- $\delta^{15}\text{N}$  in shallow-water scleractinian corals has been shown to reflect the  $\delta^{15}\text{N}$  of N sources to corals. Most studies to date have focused on anthropogenic/terrestrial N input into the reefs (Erler et al., 2015; Hoegh-Guldberg et al., 2004; Jupiter et al., 2008; Marion et al., 2005). However, the water over many reefs exchanges freely with open ocean surface waters, and the CS- $\delta^{15}\text{N}$  in corals from these reefs is expected to reflect the  $\delta^{15}\text{N}$  of open ocean N supply, an expectation that is supported by some recent data (Yamazaki et al., 2011; Wang et al., 2015). If this applies generally, then it would expand the range of potential applications of CS- $\delta^{15}\text{N}$  to studies of past changes in the open ocean N cycle, on timescales ranging from recent centuries to the distant geological past. In this study, using corals from 8 globally distributed sites, we test the hypothesis that CS- $\delta^{15}\text{N}$  of corals proximal to the open ocean is controlled by the  $\delta^{15}\text{N}$  of oceanic nitrate supplied to the euphotic zone adjacent to the reefs.

## 2. Materials and methods

### 2.1. Corals

The coral samples used in this study are from the following sites (Fig. 1; Table 1): Bermuda in the North Atlantic, the Brazil margin in the South Atlantic, the Oman margin in the Indian Ocean, the northern Great Barrier Reef (GBR), New Caledonia, the Dongsha atoll and Green Island in the western Pacific, and Kiritimati Island in the central equatorial Pacific (CEP). The sampling was proximal to the open ocean, and there is no distinctively large terrestrial input to any of these sites, increasing the likelihood that the corals directly reflect the  $\delta^{15}\text{N}$  of oceanic N sources. We have measured CS- $\delta^{15}\text{N}$  from such locations as the inshore Great Barrier Reef (Erler et al., 2015) and lagoon patch reefs nearby the

islands of Bermuda (Wang et al., 2015), but these are not included in our analysis. From the Bermuda pedestal, we use only the off-shore corals that we have measured (Wang et al., 2015). At each site, coral heads from one or multiple colonies were collected from living corals by scuba divers. Collection information for each site is given in Table 1 and supplementary Fig. 1. The Pacific and Indian Ocean coral samples in this study are *Porites* sp., except for the New Caledonia coral (*Isopora palifera*); while the Atlantic coral samples include three species: *Diploria labyrinthiformis* for Bermuda; and *Mussismilia hispida* and *Madracis decactis* for Brazil Margin. Despite the species differences, all the corals used in this study are symbiotic (i.e. have zooxanthellae).

In the lab, a slab was cut from each coral head for *Porites* and *Diploria*. The *Porites* and *Diploria* slabs were rinsed with deionized water, dried, and then scanned by computed axial tomography (CAT) to determine the maximum growth axis. Age models of the *Porites* and *Diploria* slabs were determined by counting annual growth bands in CAT scan images or by correlating with Sr/Ca records in the same core. Along the maximum growth axis, powder samples were drilled out from each slab/piece at annual/seasonal resolution. For coral species other than *Porites* and *Diploria*, no age model was generated. Tissue from these corals was removed with a jet of deionized water. Then skeleton pieces were cut from the coral skeleton with a rotary tool and diamond-coated cutting wheel. The skeleton pieces were rinsed with deionized water, dried and crushed into fine powder with agate mortar and pestle. These pieces were estimated to represent several years' growth. Table 1 describes the samples accumulated into the average CS- $\delta^{15}\text{N}$  reported for each site.

### 2.2. CS- $\delta^{15}\text{N}$ measurements

The coral carbonate powders were analyzed for CS- $\delta^{15}\text{N}$  following the protocol in Wang et al. (2015). First, in an oxidative cleaning step, 10 mL sodium hypochlorite (10–15% available chlorine) is added to 5–10 mg of coral powder in 15 mL centrifuge tubes. These centrifuge tubes are placed on an orbital shaker for 24 hrs. The oxidative cleaning step has been demonstrated to be important for the removal of contaminant organic matter (Hendy et al., 2012; Ingalls et al., 2003; Ramos-Silva et al., 2013) and thus the analysis of CS- $\delta^{15}\text{N}$  (Erler et al., 2016; Wang et al., 2015). The cleaning reagent is decanted, and the sample is rinsed 3 times with deionized water by centrifugation and decanting and then dried at 60 °C. Once dry, the sample is weighed into a 4 mL borosilicate glass vial (precombusted for 5 hrs at 500 °C) and dissolved by reaction with 4 N HCl. After dissolution, an aliquot of 1 mL freshly combined persulfate oxidizing reagent (1 g recrystallized low-N potassium persulfate and 2 g ACS grade NaOH in 100 mL deionized water) is added, and the sample is autoclaved for 1.5 hr to completely oxidize to nitrate the organic nitrogen released during decalcification. After oxidation, the sample is centrifuged; the clear supernatant is transferred to another precombusted 4 mL borosilicate glass vial and the pH of the supernatant is adjusted to near 7 with HCl and NaOH. The nitrate concentration of the sample solution is analyzed by chemiluminescence (Braman and Hendrix, 1989), mostly to determine aliquot volumes for  $\delta^{15}\text{N}$  measurement. The  $\delta^{15}\text{N}$  of the nitrate is measured by conversion to  $\text{N}_2\text{O}$  with the “denitrifier method” (Sigman et al., 2001) followed by extraction, purification, and isotopic analysis of the  $\text{N}_2\text{O}$  product (Casciotti et al., 2002). Amino acid reference materials USGS 40 and 41 are used in each batch of analyses to correct for the reagent and operational blanks, which is typically less than 2% of the total N content in an oxidized sample. An in-house coral standard (CBS-1) provides a metric for reproducibility both within an analysis batch and across batches. The analytical precision (1sd) of our protocol is 0.2‰ (Wang et al., 2015). For each coral core, an average CS- $\delta^{15}\text{N}$  ( $\pm 1\text{sd}$ ) is calcu-

**Table 1**  
Sample information and  $\delta^{15}\text{N}$  data.

Ocean region	Coral location	Coral core/colony #	Latitude	Longitude	Depth (m)	Coral species	Year of collection	Number of coral samples analyzed in each core	Sampling time-scale	Core-average skeletal $\delta^{15}\text{N}$ (‰, $\pm 1\sigma$ )	Site-average skeletal $\delta^{15}\text{N}$ (‰, $\pm 1\sigma$ )	$\delta^{15}\text{N}$ of oceanic N supply to the reefs (‰, $\pm 1\sigma$ )	References for the $\delta^{15}\text{N}$ of oceanic N supply
North Atlantic	Bermuda <sup>a</sup>	1	32.46 N	64.83 W	10	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	3.7 $\pm$ 0.5	4.1 $\pm$ 0.50	2.5 $\pm$ 0.2	Knapp et al., 2005
		2	32.46 N	64.83 W	10	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	3.9 $\pm$ 0.4			
		3	32.46 N	64.83 W	10	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	3.5 $\pm$ 0.5			
		4	32.40 N	64.79 W	4	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	4.4 $\pm$ 0.5			
		5	32.40 N	64.79 W	4	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	4.5 $\pm$ 0.2			
		6	32.40 N	64.79 W	4	<i>Diploria labyrinthiformis</i>	2005	10	annual, 1995–2005	4.7 $\pm$ 0.5			
South Atlantic	Brazil margin <sup>b</sup>	1	23.78 S	45.13 W	4–6	<i>Mussismilia hispida</i>	2013	2	multiyear	8.3 $\pm$ 0.2	8.8 $\pm$ 0.8	6.8 $\pm$ 0.2	Frame, 2011; Smart et al., 2015
Central Equatorial Pacific <sup>c</sup>	Kiritimati Island	1	1.87 N	157.40 W	9	<i>Madracis decactis</i> <i>Porites. sp</i>	2013 1998	2 20	multiyear annual, 1977–1997	9.4 $\pm$ 0.1 13.4 $\pm$ 0.5	13.4 $\pm$ 0.5	11.0 $\pm$ 0.5/ 16.2 $\pm$ 1.8	Rafter and Sigman, 2016
South Pacific	Northern Great Barrier Reef	1	12.38 S	143.74 E	3–5	<i>Porites. sp</i>	1990	30	semi-annual, 1975–1990	5.9 $\pm$ 0.2	6.2 $\pm$ 0.4	6.1 $\pm$ 0.2	Yoshikawa et al., 2015
		2	13.33 S	143.96 E	3–5	<i>Porites. sp</i>	1990	32	semi-annual, 1974–1990	6.6 $\pm$ 0.3			
	New Caledonia	1	20.42 S	164.03 E	5	<i>Isopora palifera</i>	1995	2	multiyear	6.2 $\pm$ 0.3	6.2 $\pm$ 0.3	6.1 $\pm$ 0.2	Yoshikawa et al., 2015
North Pacific	Green Island	1	22.65 N	121.47 E	6	<i>Porites. sp</i>	2013	18	seasonal, 2009–2013	4.2 $\pm$ 0.6	4.2 $\pm$ 0.6	3.9 $\pm$ 1.0	Ren et al., unpublished data
South China Sea	Dongsha Atoll	1	20.76 N	116.79 E	1	<i>Porites. sp</i>	2013	18	seasonal, 2009–2013	5.7 $\pm$ 0.5	5.9 $\pm$ 0.5	5.5 $\pm$ 0.3	Ren et al., unpublished data
		2	20.70 N	116.89 E	1	<i>Porites. sp</i>	2013	18	seasonal, 2009–2013	5.6 $\pm$ 0.4			
		3	20.74 N	116.75 E	4	<i>Porites. sp</i>	2013	18	seasonal, 2009–2013	6.5 $\pm$ 0.3			
Arabian Sea	Oman margin	1	17.50 N	55.7 E	3	<i>Porites. sp</i>	1996	12	seasonal, 1984–1985 & 1993–1995	10.1 $\pm$ 0.2	10.1 $\pm$ 0.20	9.0 $\pm$ 1.0	Brandes et al., 1998

<sup>a</sup> At Bermuda, 10 coral cores/colonies from 4 sites were analyzed for skeletal  $\delta^{15}\text{N}$ , but only the two offshore sites-data are shown here because we seek to compare the skeletal  $\delta^{15}\text{N}$  to the  $\delta^{15}\text{N}$  of open ocean nitrate supplied to the reefs. Please refer to Wang et al. (2015) for further details.

<sup>b</sup> At the Brazil margin, two species of corals adjacent to each other were analyzed for skeletal  $\delta^{15}\text{N}$ .

<sup>c</sup> In the central equatorial Pacific, the surface nitrate is only partially consumed; thus, both nitrate assimilation and feeding may contribute N to the Kiritimati coral. Here, the coral skeletal  $\delta^{15}\text{N}$  is compared with both the mixed layer nitrate  $\delta^{15}\text{N}$  (16.2  $\pm$  1.8‰) at a set of stations adjacent to Kiritimati (155°W, 0–1°N) and the  $\delta^{15}\text{N}$  of instantaneously produced PON (11.0  $\pm$  0.5‰) calculated from Rayleigh model. Please refer to Fig. 3 for more details.

lated from the time-series data. For each site, an average CS- $\delta^{15}\text{N}$  ( $\pm 1\text{sd}$ ) is calculated from all coral cores (Table 1).

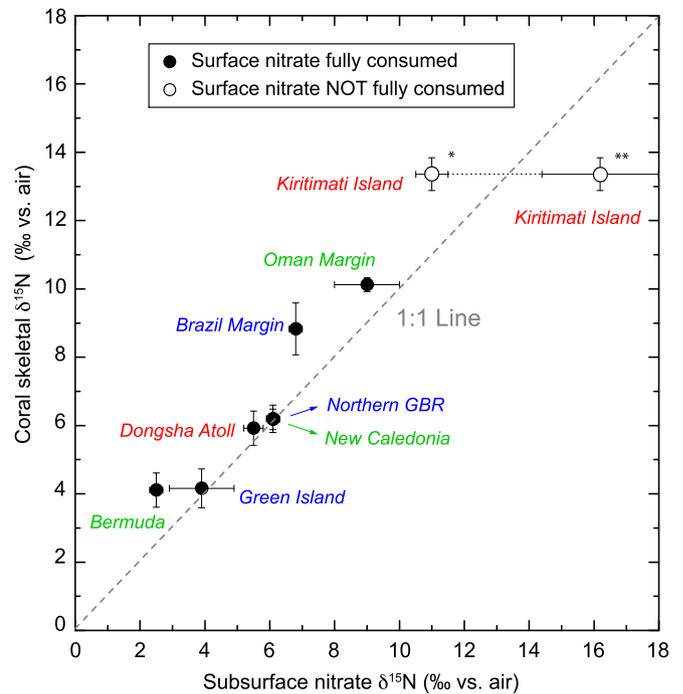
### 3. Results

At each site, CS- $\delta^{15}\text{N}$  shows relatively weak temporal and spatial variability ( $1\text{sd} < 1\text{‰}$ , Table 1), with a range of  $< 3\text{‰}$  in any single core (Table 1). The difference in CS- $\delta^{15}\text{N}$  between species is only  $1.1\text{‰}$  at Brazil margin, consistent with a previous study off-shore of Heron Island in the Great Barrier Reef suggesting a difference between two coral species of  $1.1\text{‰}$  (Erler et al., 2015). In contrast, among the 8 sites, we observe a broad range of  $\sim 10\text{‰}$  in CS- $\delta^{15}\text{N}$ , with the highest values on Kiritimati Island ( $13.4 \pm 0.5\text{‰}$ ) and the lowest values on Bermuda ( $4.1 \pm 0.5\text{‰}$ ) and Green Island ( $4.2 \pm 0.6\text{‰}$ ). The Brazil Margin corals and Oman margin corals show a relatively high CS- $\delta^{15}\text{N}$  of  $8.8 \pm 0.8\text{‰}$  and  $10.1 \pm 0.2\text{‰}$ , respectively. The Northern Great Barrier Reef, New Caledonia and Dongsha atoll corals show a similar CS- $\delta^{15}\text{N}$  of  $6.2 \pm 0.6\text{‰}$ ,  $6.2 \pm 0.3\text{‰}$ , and  $5.9 \pm 0.5\text{‰}$ , respectively.

### 4. Interpretation and discussion

Even without considering global warming and ocean acidification, anthropogenic impacts threaten many coral reefs (Hughes et al., 2003). One major anthropogenic factor is the increasing nutrient transport into inshore reefs (e.g., inshore South Great Barrier Reef; Brodie et al., 2011). For this reason, coral  $\delta^{15}\text{N}$  studies have often focused on tracking anthropogenic/terrestrial N inputs to coral reefs (Baker et al., 2010; Erler et al., 2015; Jupiter et al., 2008; Marion et al., 2005; Yamazaki et al., 2011). However, many coral reefs are far from terrestrial N sources and must rely on N supplied from the open ocean. It is thus expected that the CS- $\delta^{15}\text{N}$  of these corals should reflect the  $\delta^{15}\text{N}$  of oceanic N sources. Below, we compare our CS- $\delta^{15}\text{N}$  results with the  $\delta^{15}\text{N}$  of oceanic N supplied to the reef from which each coral derives.

In laboratory experiments, symbiotic corals have been demonstrated to access N from a range of sources, including particulate organic matter and plankton ingestion (Houlbrèque and Ferrier-Pagès, 2009) as well as ammonium and nitrate assimilation (Badgley et al., 2006; Grover et al., 2002; Tanaka et al., 2006). However, in the real ocean, symbiotic corals in a given region likely rely on a more limited set of N forms, depending on availability. For example, in oligotrophic waters such as around Bermuda, the surface ocean concentrations of ammonium and nitrate are less than  $20\text{ nM}$  (Fawcett et al., 2014; Lomas et al., 2013), well below the assimilation thresholds found in lab experiments (Badgley et al., 2006; Tanaka et al., 2006). In such waters, the ultimate N source is dominantly the nitrate from wintertime deep mixing or upwelling (Altabet, 1988; Knapp et al., 2005), while the N available to the corals is mostly in organic forms that were produced from this nitrate by phytoplankton assimilation, followed by upper ocean N cycling and the phytoplankton assimilation of ammonium. In contrast, in upwelling systems such as the central and eastern equatorial Pacific, surface nitrate concentrations can be high. As a result, symbiotic corals in such systems might use nitrate from the open ocean or organic N produced over the reef or imported from the open ocean. Accordingly, we first divide the 8 coral sites in this study into two categories based on whether the adjacent surface ocean nitrate is fully consumed. Among our 8 coral sites, only the CEP coral site (Kiritimati Island) is from an ocean region where the mixed layer nitrate is frequently at micromolar levels. Even at the Oman site, which hosts seasonal offshore upwelling associated with the Indian summer monsoons, there is rarely significant unused nitrate in the waters over the reef or immediately offshore (World Ocean Atlas 2013). Thus, we group it with the corals from reefs with low



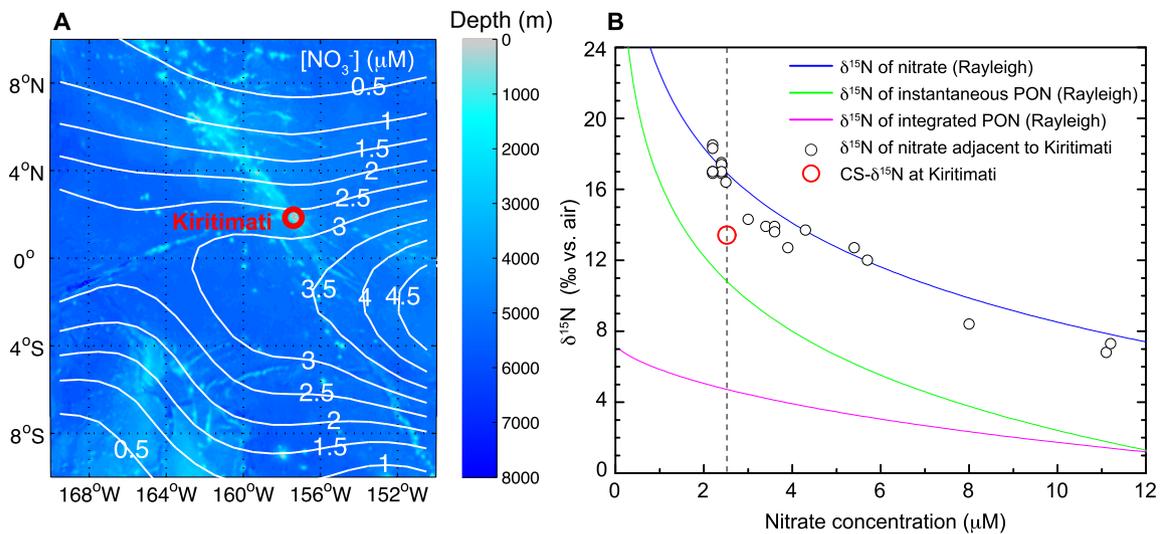
**Fig. 2.** Comparison of site-average coral skeletal  $\delta^{15}\text{N}$  (CS- $\delta^{15}\text{N}$ ) with subsurface nitrate  $\delta^{15}\text{N}$ . The annual mean surface nitrate concentrations in the adjacent open ocean are below  $0.5\text{ }\mu\text{M}$  for all the corals sites (filled circles) except for Kiritimati Island (open circles). Thus, subsurface nitrate  $\delta^{15}\text{N}$  is used as the x-axis parameter for all corals sites except for Kiritimati Island. In the central equatorial Pacific, the surface nitrate is not fully consumed; thus, both nitrate and PON are available to Kiritimati corals as their potential N sources. As it is unclear whether corals rely on nitrate assimilation or feeding as their primary N sources at Kiritimati, both the  $\delta^{15}\text{N}$  of instantaneous PON (labeled with \*) as calculated from the Rayleigh model (Fig. 3) and the average  $\delta^{15}\text{N}$  of mixed layer nitrate measured at a set of stations adjacent to Kiritimati ( $155^{\circ}\text{W}$ ,  $0\text{--}1^{\circ}\text{N}$ , labeled with \*\*, Fig. 3) are used as the x-axis parameter (Rafter and Sigman, 2016). The y-axis errors (1sd) are calculated from the CS- $\delta^{15}\text{N}$  data at each site while the x-axis errors (1sd) are calculated from available nitrate  $\delta^{15}\text{N}$  data (Table 1). With regard to the latter, the actual uncertainty is likely greater due to spatial and temporal variation.

surface nitrate concentrations; this decision is discussed further in Section 4.2.

#### 4.1. Corals from low nutrient ocean regions

In ocean regions where the annual mean mixed layer concentration of inorganic N is less than roughly  $0.5\text{ }\mu\text{M}$ , the assimilation of inorganic N by corals is probably too slow to be significant, especially when food is available (Grover et al., 2002). Only in experiments where corals are starved is there evidence for significant nitrate/ammonium uptake, and then mostly at concentrations above  $\sim 2\text{ }\mu\text{M}$  (Badgley et al., 2006; Tanaka et al., 2006). If this view of inorganic N uptake is correct, corals in most tropical ocean regions must rely dominantly on organic matter as their N source. The  $\delta^{15}\text{N}$  of euphotic zone biomass in these regions is expected to covary with the  $\delta^{15}\text{N}$  of subsurface nitrate (immediately below the euphotic zone), which reflects the  $\delta^{15}\text{N}$  of the nitrate supply to the euphotic zone, and the available data support this expectation (Fawcett et al., 2011; Graham et al., 2010; Lorrain et al., 2015; Montoya et al., 2002; Ren et al., 2012). Thus, for all our coral sites except the CEP site, we compare CS- $\delta^{15}\text{N}$  to the  $\delta^{15}\text{N}$  of subsurface nitrate (Fig. 2).

It is more challenging to address which specific fractions of particulate organic matter in the water column are accessed by corals. At Bermuda, the  $\delta^{15}\text{N}$  of bulk suspended particulate organic nitrogen (PON) in the surface ocean is  $< 0\text{‰}$ , as is the  $\delta^{15}\text{N}$  of the



**Fig. 3.** (A) Bathymetric map of central equatorial Pacific and our coral site (Kiritimati Island). White contours are climatological annual mean mixed layer nitrate concentrations (World Ocean Atlas 2013). (B) Rayleigh model for nitrate consumption in the central equatorial Pacific (Initial condition:  $[\text{NO}_3^-] = 12.4 \mu\text{M}$ ; nitrate  $\delta^{15}\text{N} = 7.2\text{‰}$ ; isotope effect =  $6.0\text{‰}$  (Rafter and Sigman, 2016)). The blue, green and purple lines correspond to the residual nitrate, instantaneous PON and integrated PON, respectively. The vertical dashed line corresponds to the climatological annual mean mixed layer nitrate concentration adjacent to the coral site. Open black circles denote nitrate  $\delta^{15}\text{N}$  and concentration measurements from 0 to 150 m at a set of stations close to Kiritimati (155°W, 0–1°N) (Rafter and Sigman, 2016).

dominant prokaryotic phytoplankton (Fawcett et al., 2011). However, larger biological particles such as eukaryotic phytoplankton and zooplankton are higher in  $\delta^{15}\text{N}$  (Fawcett et al., 2011; Montoya et al., 2002), and many investigators would argue that these are more important prey items for corals (Houlbrèque and Ferrier-Pagès, 2009). At Bermuda near Hog Reef (a northern fringing reef), the average  $\delta^{15}\text{N}$  of net tow-collected plankton larger than  $35 \mu\text{m}$  is  $3.5\text{--}4\text{‰}$  (Wang et al., 2015), similar to the CS- $\delta^{15}\text{N}$  at this location (Table 1). A study of two coral species suggested that CS- $\delta^{15}\text{N}$  was indistinguishable from the coral tissue  $\delta^{15}\text{N}$  in one species and  $1\text{--}1.5\text{‰}$  higher than the coral tissue  $\delta^{15}\text{N}$  in the other (Erlor et al., 2015). The CS- $\delta^{15}\text{N}$ /tissue  $\delta^{15}\text{N}$  offset and any variations among species are of interest. In any case, the data from Hog Reef imply that coral tissue  $\delta^{15}\text{N}$  is similar to (not clearly higher than) the  $\delta^{15}\text{N}$  of their food source. This fits into a broader interpretation of coral  $\delta^{15}\text{N}$  data (Wang et al., 2015), in which corals recycle their metabolic ammonium to their symbionts (Falkowski et al., 1993; Kopp et al., 2013; Tanaka et al., 2006), reducing their elevation in  $\delta^{15}\text{N}$  relative to their food source below the  $\sim 3.5\text{‰}$  typical of heterotrophs (Minagawa and Wada, 1984).

The shallow-water scleractinian corals used in this study are from regions that cover a range of subsurface nitrate  $\delta^{15}\text{N}$ , due to the hydrography and biogeochemistry of each ocean region. The Sargasso Sea and South China Sea coral sites are characterized by low subsurface nitrate  $\delta^{15}\text{N}$ , primarily due to N fixation (Kao et al., 2012; Knapp et al., 2008). The Arabian Sea coral site has a high subsurface nitrate  $\delta^{15}\text{N}$ , apparently caused by mixing with the underlying water column denitrification zones, which preferentially remove  $^{14}\text{N}$ -nitrate relative to  $^{15}\text{N}$ -nitrate and leave the residual nitrate enriched in  $^{15}\text{N}$  (Brandes et al., 1998). The South Atlantic coral site is characterized by a subsurface nitrate  $\delta^{15}\text{N}$   $1\text{--}2\text{‰}$  higher than deep ocean nitrate  $\delta^{15}\text{N}$  (Frame, 2011; Smart et al., 2015), which is attributed to incomplete nitrate consumption in the Southern Ocean and northward transport of Subantarctic Mode Water and Antarctic Intermediate Water (Smart et al., 2015). The northern Great Barrier Reef and New Caledonia sites appear to have a subsurface nitrate  $\delta^{15}\text{N}$  of  $6.1\text{‰}$ , slightly higher than the deep ocean nitrate  $\delta^{15}\text{N}$  in the same region (Yoshikawa et al., 2015).

#### 4.2. Corals from nitrate-rich upwelling ocean regions

In the eastern and central equatorial Pacific, easterly trade winds drive upwelling of cool and nutrient-rich waters. Phytoplankton assimilate only a portion of the upwelled nitrate at the equator, with the remaining nitrate being drawn down as it is advected off axis (Fig. 3). Due to isotope fractionation during nitrate assimilation, the decline in nitrate concentration from the subsurface into the surface and northward and southward from the equator is accompanied by a rise in the  $\delta^{15}\text{N}$  of nitrate, following the path of the upwelled water parcel (Altabet and Francois, 1994; Rafter et al., 2012). In the CEP, the surface water is dominantly sourced from the underlying Equatorial Under Current (EUC), which has a nitrate concentration of  $12.4 \pm 2.5 \mu\text{M}$  and a nitrate  $\delta^{15}\text{N}$  of  $7.2 \pm 0.3\text{‰}$  (Rafter and Sigman, 2016). Most data from the CEP indicate the consumption of nitrate without resupply subsequent to upwelling into the euphotic zone, consistent with the Rayleigh model, and the isotope effect of nitrate assimilation estimated from the Rayleigh model substrate equation is  $6.0 \pm 0.4\text{‰}$  (Rafter and Sigman, 2016).

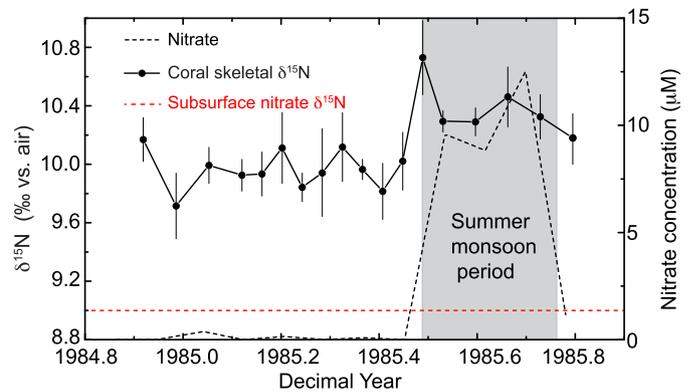
In contrast to corals from low nutrient regions, there are three potential oceanic N sources for the CEP corals, and the Rayleigh model can be used to estimate the  $\delta^{15}\text{N}$  of these N sources given the isotopic parameters described above and the climatological annual mean mixed layer nitrate concentrations (Fig. 3): (1) surface water nitrate (from the substrate equation of the Rayleigh model); (2) euphotic zone PON accumulated since the time of upwelling (from the integrated product of the Rayleigh model); and PON generated over the reefs from mixed layer nitrate imported from the open ocean (from the instantaneous product of the Rayleigh model). Because of the limited data on these reefs, it is challenging to identify the dominant N sources to the corals. Among the three N sources described above, we expect that the Kiritimati coral obtains its N primarily by feeding on the PON generated on the reef as the nitrate is consumed. Several lines of evidence support this hypothesis. The CS- $\delta^{15}\text{N}$  data from Kiritimati Island coral ( $13.4 \pm 0.5\text{‰}$ ) are closest to the  $\delta^{15}\text{N}$  of PON expected from the instantaneous product of the Rayleigh model ( $\sim 11\text{‰}$ , Fig. 3), given the open ocean euphotic zone nitrate concentrations adjacent to the reefs as well as the available Kiritimati

reef nitrate/nitrite concentration data (Fig. 3) (Dinsdale et al., 2008; Rafter and Sigman, 2016). In addition, Kiritimati Island sits at 2°N, outside of the core upwelling zone near the equator. It is unlikely that PON produced since the time of upwelling (as would be approximated by the integrated product of the Rayleigh model) can travel so far north without being exported to depth. Further, given prior data on the relationship between co-occurring PON  $\delta^{15}\text{N}$  and CS- $\delta^{15}\text{N}$  (Wang et al., 2015; Erler et al., 2015), feeding on PON accumulated in the open ocean euphotic zone, as simulated by the integrated product of the Rayleigh model, would be expected to yield a CS- $\delta^{15}\text{N}$   $\sim 9\%$  lower than we measured.

Corals are capable of assimilating nitrate (Badgley et al., 2006; Tanaka et al., 2006), and the process is often elicited in laboratory studies by starving corals, that is, foregoing purposeful feeding. There are almost certainly oceanic conditions under which corals make use of nitrate assimilation. However, we consider it unlikely that nitrate assimilation is the primary N source for corals at Kiritimati. Nitrate taken up by the coral symbiotic system is assimilated into biomass by the zooxanthellae, and yet the zooxanthellae cytoplasm is separated from ambient water by 3 membranes or more. This provides a basic mechanistic reason that corals would undertake minimal nitrate assimilation under low nitrate concentrations and/or when PON availability is high. Moreover, because nitrate must efflux back into the environment for nitrate assimilation to drive significant isotope fractionation (Karsh et al., 2014; Needoba et al., 2004), the multiple cell boundaries lead to the expectation that isotope fractionation during nitrate assimilation will be low, especially at low nitrate concentrations. Indeed, this effect has been observed in culture experiments, such that at a seawater nitrate concentration relevant to Kiritimati Island (i.e. 2–3  $\mu\text{M}$ ), the isotope effect of nitrate assimilation is only  $\sim 2\%$  (Devlin, 2015). Complete reliance on coral nitrate assimilation could be argued to be inconsistent with the observation that the nitrate  $\delta^{15}\text{N}$  is 3–4‰ higher than the CS- $\delta^{15}\text{N}$  (Fig. 3). Nevertheless, given the uncertainties in this comparison, we cannot rule out the possibility of dominant or partial reliance on nitrate assimilation. The combined occurrence of nitrate assimilation and feeding by Kiritimati corals is clearly possible on the basis of the isotopic comparison, as CS- $\delta^{15}\text{N}$  falls in between the  $\delta^{15}\text{N}$  of these two N sources (Fig. 3).

Because the subsurface nitrate supply is only partially consumed in CEP surface waters, the  $\delta^{15}\text{N}$  of the subsurface nitrate near Kiritimati is not an appropriate measure of the  $\delta^{15}\text{N}$  of the N available to the coral. Accordingly, in Fig. 2, the parameter applied to the x-axis for the Kiritimati coral is different from that of the other 7 sites. Because of the ambiguity associated with feeding versus nitrate assimilation, we show symbols for both the average  $\delta^{15}\text{N}$  of mixed layer nitrate at a set of stations (155°W, 0–1°N) adjacent to Kiritimati and the calculated  $\delta^{15}\text{N}$  of instantaneous PON as the x-axis parameter in Fig. 2. To indicate this difference in x-axis parameter in Fig. 2, the Kiritimati coral is indicated with two open (rather than filled) symbols.

It should be asked whether the above considerations are applicable to reefs adjacent to coastal upwelling such as the Oman margin coral. The open ocean surface waters off the Oman margin are strongly influenced by the Indian Monsoon system. During the summer (late June to early October), the southwestern monsoon induces Ekman upwelling, with non-zero nitrate during the summer upwelling period (World Ocean Atlas 2013). During the rest of the year (middle October to middle June), nitrate drops to oligotrophic open ocean levels (Fig. 4). If nitrate assimilation in the waters overlying the Oman margin coral is an important source of PON to the coral, based on the instantaneous product equation of the Rayleigh model, a summertime decrease of  $\sim 2\%$  would be expected in the coral  $\delta^{15}\text{N}$ . In contrast, a seasonally resolved Oman margin CS- $\delta^{15}\text{N}$  record suggests very little ( $\sim 0.5\%$ ) variation, with slightly higher CS- $\delta^{15}\text{N}$  observed in the summer (Fig. 4). Moreover,



**Fig. 4.** Monthly variation in coral skeletal  $\delta^{15}\text{N}$  and adjacent open ocean mixed layer nitrate concentration (climatology) over a one-year window from near Oman in the Arabian Sea. Nitrate concentration data from open ocean waters adjacent to the coral site are from World Ocean Atlas 2013. Shaded area indicates the southwest monsoon period (late June to early October). Red dashed line indicates the subsurface nitrate  $\delta^{15}\text{N}$  in the adjacent Arabian Sea (Brandes et al., 1998). The uncertainty of the coral chronology is estimated to be 2 months. The CS- $\delta^{15}\text{N}$  change is small ( $\sim 0.5\%$ ) and in the opposite sense expected if the reef were considered to be bathed by nitrate-rich waters. For this and other reasons, the Oman coral was grouped with the corals from open ocean settings of regionally complete nitrate consumption. One possible explanation for the weak rise in CS- $\delta^{15}\text{N}$  during the summer monsoon period is that rapid feeding by the coral outpaced the ammonium assimilation of the symbionts, leading to efflux of low  $\delta^{15}\text{N}$  ammonium and a slight rise in the  $\delta^{15}\text{N}$  of the coral host/symbiont system (Wang et al., 2015). However, lacking further information, other possibilities cannot be ruled out.

as described below, the CS- $\delta^{15}\text{N}$  of the Oman coral is consistent with the relationship observed among the corals from oligotrophic sites (Fig. 2). These observations suggest that open ocean-produced PON is the dominant N source to the Oman margin reefs. The difference from the CEP coral is consistent with the high productivity in the Oman upwelling and complete consumption of nitrate prior to transport of the open ocean waters onto the Oman margin. While we cannot be confident that the same situation will apply across all coastal upwelling systems, this would appear likely: coastal upwelling systems typically involve consumption of the upwelled nutrients near the site of upwelling, and lateral transport of any residual nutrients is most often offshore rather than onshore.

#### 4.3. Global comparison of CS- $\delta^{15}\text{N}$ to the subsurface nitrate $\delta^{15}\text{N}$

Comparing CS- $\delta^{15}\text{N}$  to the subsurface nitrate  $\delta^{15}\text{N}$ , we observe a strong linear correlation ( $R^2 = 0.82$ , Fig. 2). The average difference between CS- $\delta^{15}\text{N}$  and the subsurface nitrate  $\delta^{15}\text{N}$  (excluding Kiritimati Island) is  $0.8 \pm 0.8\%$ . These values indicate that CS- $\delta^{15}\text{N}$  is only slightly higher than the  $\delta^{15}\text{N}$  of subsurface nitrate supplied to the reefs. This observation is consistent with previous findings that (1) corals feed on a PON/plankton pool that resembles the  $\delta^{15}\text{N}$  of the nitrate supplied to and consumed in the euphotic zone, and (2) symbiotic corals do not exhibit the  $\sim 3\%$  trophic  $\delta^{15}\text{N}$  elevation because of minimal loss of low- $\delta^{15}\text{N}$  ammonium to the oligotrophic waters (Reynaud et al., 2009; Wang et al., 2015). From a paleoceanographic perspective, the strong correlation indicates that, for corals that are proximal to open water, the subsurface nitrate  $\delta^{15}\text{N}$  is the dominant control on CS- $\delta^{15}\text{N}$ .

#### 4.4. Implications for paleoceanography

The strong correlation between the CS- $\delta^{15}\text{N}$  and the  $\delta^{15}\text{N}$  of subsurface nitrate suggests that CS- $\delta^{15}\text{N}$  will be a powerful tool for studying the past marine N cycle. Ocean sediments are the primary existing archives for reconstructing marine N cycling in the past. Bulk sedimentary  $\delta^{15}\text{N}$ , microfossil-bound  $\delta^{15}\text{N}$  and compound-specific  $\delta^{15}\text{N}$  from sediments have all contributed to our under-

standing of the past marine N cycle. Relative to these sediment-based archives, shallow-water scleractinian corals have the potential to record orders of magnitude higher temporal resolution, due to substantial skeletal growth rates and the lack of a smoothing process akin to the bioturbation of sediments. Direct absolute age dating as well as annual band counting will improve the dating of N cycle changes and allow for N cycle-focused studies of short time scale phenomena such as El Niño-Southern Oscillation.

Deep-sea corals (both proteinaceous corals and scleractinian corals) have also been explored as an archive for studying the past marine N cycle (Sherwood et al., 2014; Wang et al., 2014). In comparison to proteinaceous corals, scleractinian corals have the advantage that the carbonate skeleton can protect the CSOM for hundreds of millions of years, and more broadly, diagenesis is less of a concern. In comparison to the deep-sea corals, shallow-water scleractinian corals have the advantages that the samples are widely accessible and that the potential time resolution of the records is much higher.

One possible complication of using CS- $\delta^{15}\text{N}$  as a N cycle proxy is the previously suggested effect of feeding rate on the coral/symbiont N cycle and its consequences for the  $\delta^{15}\text{N}$  of the coral system (Wang et al., 2015). It was observed that in highly productive inshore waters of Bermuda pedestal, the CS- $\delta^{15}\text{N}$  is  $\sim 3\text{‰}$  higher than that of the Bermuda outer reef corals discussed here, with  $\sim 2\text{‰}$  of this difference deriving from a change in CS- $\delta^{15}\text{N}$  relative to the  $\delta^{15}\text{N}$  of its N source. This finding was explained as the result of ammonium leakage out of the coral symbiotic system when corals increase their feeding rates in the highly productive inshore waters.

For corals that are proximal to the oligotrophic tropical and subtropical ocean, the productivity effect is probably minor (e.g., in the case of offshore corals at Bermuda). Indeed, the strength of correlation between CS- $\delta^{15}\text{N}$  and the  $\delta^{15}\text{N}$  of nitrate consumed across the corals studied to date (Fig. 2) suggests that variation in “baseline  $\delta^{15}\text{N}$ ” overshadows other processes in setting the CS- $\delta^{15}\text{N}$  of a given coral. Further modern studies that benefit from robust information on environmental variables at a study site (e.g., water column productivity, nutrient concentrations) may allow the individual effects on CS- $\delta^{15}\text{N}$  of source  $\delta^{15}\text{N}$  and productivity to be diagnosed more completely. In coral cores, a correlation between CS- $\delta^{15}\text{N}$  and coral extension rate is observed among the Bermuda corals (Logan et al., 1994; Wang et al., 2015), which might allow for attribution of certain downcore CS- $\delta^{15}\text{N}$  changes to the tightness of the coral’s internal N recycling as opposed to baseline  $\delta^{15}\text{N}$ . More generally, parallel measurements of CS- $\delta^{15}\text{N}$  and other proxies (e.g., coral extension rate,  $\delta^{13}\text{C}$ ,  $\delta^{11}\text{B}$ ) could yield complementary information that will compensate for the uncertainties of individual proxies.

In the near term, we consider it simpler to interpret CS- $\delta^{15}\text{N}$  from coral sites near reef margins in nutrient-poor ocean regions, where there is not the potential for variable, large scale inputs of oceanic nitrate onto the reef and where feeding changes are less likely to drive coral host/symbiont effects on CS- $\delta^{15}\text{N}$ . In the same sense, CS- $\delta^{15}\text{N}$  is in the greatest need of further ground-truthing in nutrient-rich oceanic regions and/or in inshore reef systems.

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## Appendix A. Supplementary material

Supplementary material related to this article can be found online at <http://dx.doi.org/10.1016/j.epsl.2016.02.032>.

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