

Consistency in coral skeletal amino acid composition offshore of Palau in the western Pacific warm pool indicates no impact of decadal variability in nitricline depth on primary productivity

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Abstract

The depth of the thermocline and associated nitricline in the western Pacific warm pool (WPWP) vary over time in response to changes in larger ocean-atmosphere climate patterns. A shoaling of the nitricline in the WPWP brings nitrate-rich seawater ($\text{NO}_3^- > 4 \mu\text{mol kg}^{-1}$) above the base of the euphotic zone, stimulating primary productivity. Here, we test if decadal variability in the nitricline depth is driving changes in regional primary productivity and source nitrate dynamics. We use the nitrogen isotopic composition ($\delta^{15}\text{N}$) of amino acids in the skeleton of a proteinaceous coral collected from the base of the euphotic zone in the WPWP. In proteinaceous corals, as in most organic life, the $\delta^{15}\text{N}$ of phenylalanine matches that of the ambient nitrate while the $\delta^{15}\text{N}$ of trophic amino acids reflect subsequent trophic transfer of the nitrogen prior to incorporation into the coral's food, suspended particulate organic matter. Consistency of the trophic position of the coral calculated from the $\delta^{15}\text{N}$ composition of the coral skeletal amino acids over its 56 yr lifespan suggest that decadal variability in nitricline depth and subsequent shifts in nitrate availability to the euphotic zone have not impacted primary productivity offshore of Palau in the WPWP. This is important when considering the current external forcing of Pacific Ocean climate patterns and the resulting impacts on the global carbon cycle in the Palau region of the WPWP.

In the western Pacific warm pool (WPWP), the barrier layer formed between the base of the shallow halocline and the top of the thermocline at the bottom of the mixed layer suppresses the upward flux of nutrients (Fig. 1) (Keeling and Revelle 1985; Lukas and Lindstrom 1991; Feely et al. 2006). This results in nutrient poor, oligotrophic surface waters. However, a slight chlorophyll maximum forms in the barrier layer at the intersection of sufficient light and nutrients for primary productivity (Matsumoto et al. 2004; Turk et al. 2011). Seasonally and during El Niño events, the eastward movement of the warm, fresh surface water combined with turbulent vertical mixing and wind-driven upwelling in the WPWP, reduces the depth of the barrier layer. This brings

seawater with nitrate higher within the euphotic zone, creating a significant sub-surface chlorophyll maximum, which alters the soft tissue carbon pump, and regional CO_2 dynamics (Omta et al. 2007; Turk et al. 2011; Gierach et al. 2012; Valsala et al. 2014). Despite this, shoaling of the $1 \mu\text{M}$ nitrate isopleth did not substantially change depth-integrated primary productivity between La Niña to El Niño events from 1994 to 2003 (Matsumoto and Furuya 2011). Thus, nitricline depth variability on interannual timescales is thought to leave primary production unaltered. The depth of the thermocline and thus nitricline also significantly varies on decadal timescales (Zhang et al. 2007). Yet, due to the lack of long term records, the impact of these decadal variations on primary productivity in the WPWP is unknown.

After the Pacific Decadal Oscillation regime shift in 1977, the walker circulation weakened (Gierach et al. 2012), causing the thermocline/nitricline to shoal in the WPWP (McPhaden and Zhang 2002; Zhang and Busalacchi 2007).

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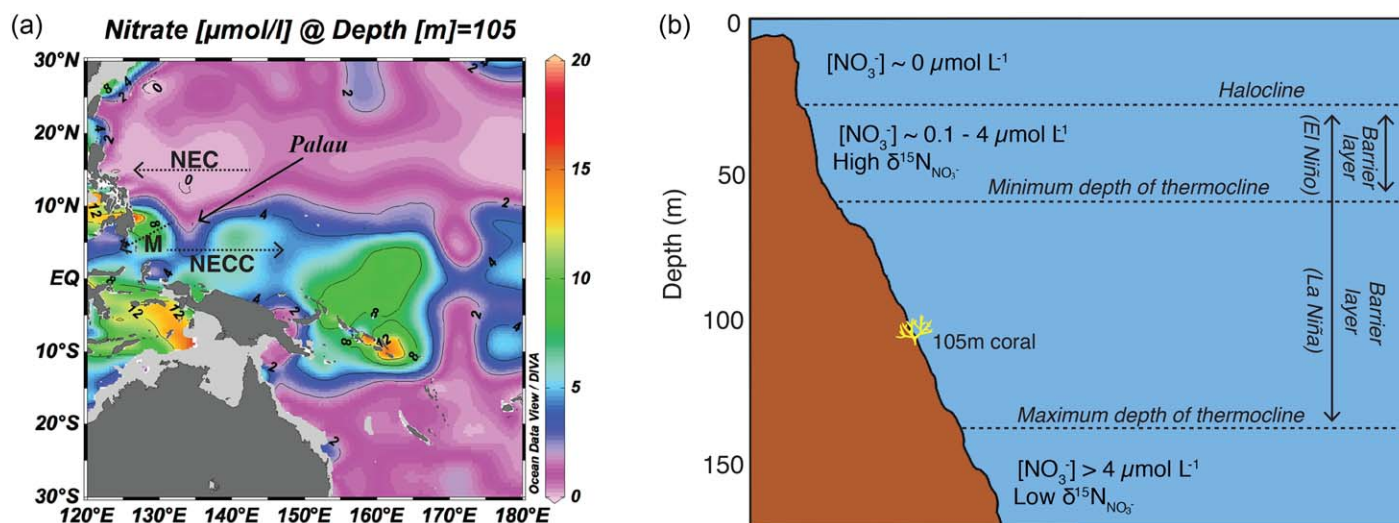


Fig. 1. Cartoon of vertical profile of nitricline depth variability, moving above and below the depth of the collected corals. NEC = North Equatorial Current, NECC = North Equatorial Countercurrent, and M = Mindanao Current. Nitrate concentrations obtained from Matsumoto et al. (2004) and Yoshikawa et al. (2006).

Since 1999, corresponding to a possible Pacific Decadal Oscillation shift, trade winds have increased and there has been a deepening the thermocline and nitricline in the WPWP (Yang and Wang 2009; Trenberth and Fasullo 2013; de Boissésou et al. 2014). This 1999 shift also corresponded to a period of increased frequency of central Pacific-El Niño events rather than the canonical eastern Pacific events (Yeh et al. 2009; Lee and McPhaden 2010). These multidecadal shifts could be part of the natural variability in the Pacific ocean-atmosphere climate system (McPhaden et al. 2011; Newman and Shin 2011), or part of a larger anthropogenic shift in our climate (Yeh et al. 2009). Regardless of the cause, the potential influence of the decadal timescale shoaling and deepening of the nitricline on primary productivity in the WPWP is unknown.

To test if primary productivity in the WPWP has varied on decadal timescales, we measured the $\delta^{15}\text{N}$ composition of individual amino acids ($\delta^{15}\text{N}_{\text{AA}}$) in the skeleton of the heterotrophic deep-sea proteinaceous coral *Muricella* sp. to test for changes in coral's trophic position. In the proteinaceous corals, skeletal $\delta^{15}\text{N}_{\text{AA}}$ serves as a proxy for the $\delta^{15}\text{N}_{\text{AA}}$ composition of its food source (Sherwood et al. 2011, 2013), which to the *Muricella* coral is suspended particulate organic matter from seawater that was produced in situ toward the base of the euphotic zone (Williams and Grottoli 2010a,b). The $\delta^{15}\text{N}_{\text{AA}}$ of the organic matter in turn reflects the $\delta^{15}\text{N}$ composition of the nitrate supporting primary productivity and subsequent isotopic enrichment of the nitrogen with trophic transfer (McClelland and Montoya 2002; McCarthy et al. 2007; Chikaraishi et al. 2009). We can separate the relative influences of the $\delta^{15}\text{N}$ of the source nitrate and subsequent $\delta^{15}\text{N}$ enrichment with trophic transfer that drive the organic

matter $\delta^{15}\text{N}_{\text{AA}}$ because the $\delta^{15}\text{N}$ composition of the trophic amino acids ($\delta^{15}\text{N}_{\text{AA-Tr}}$) are enriched in ^{15}N by 3–8‰ during trophic transfer while the $\delta^{15}\text{N}$ of the amino acid phenylalanine ($\delta^{15}\text{N}_{\text{Phe}}$) display little isotopic enrichment in ^{15}N with trophic transfer ($0.4\text{‰} \pm 0.5\text{‰}$) (Chikaraishi et al. 2015 and references therein). Thus, the $\delta^{15}\text{N}_{\text{Phe}}$ composition of the coral skeleton serves as a measure of the isotopic composition of the source nitrate to the food web at the base of the euphotic zone. The trophic position of the coral calculated from the $\delta^{15}\text{N}_{\text{AA}}$ composition determines if the ratio of autotrophic and heterotrophic-derived material contributing to the coral's food had remained constant over time. If constant, this suggests that there has been no change in autotrophic material in the POM that the coral feeds on reflecting variable phytoplankton primary productivity. As such, here we use the $\delta^{15}\text{N}$ of coral skeletal amino acids to test for decadal timescale changes in primary productivity offshore of Palau in the WPWP.

Methods

We measured the $\delta^{15}\text{N}$ of the amino acids alanine (Ala), proline (Pro), glutamic acid (Glu), valine (Val), leucine (Leu), phenylalanine (Phe), and glycine (Gly) in the skeleton of the azooxanthellate proteinaceous coral *Muricella* to reconstruct past in situ organic matter $\delta^{15}\text{N}$ composition, the ΣV parameter which is a measure of potential microbial resynthesis of organic matter, coral trophic position, and potential changes in primary production in the WPWP. The coral was collected live from 105-m depth from Short Drop Off Reef (7°16.4'N, 134°31.4'E), an underwater scarp located 2-km offshore of Palau in 2008 (Fig. 1). Thick cross-sections of the basal trunk

of the skeleton were cleaned in an ultrasonic bath and air dried. Working from the outer (youngest) to the center (oldest) part of the skeleton, coeval skeletal samples were drilled at six separate intervals using a hand-held Dremel drill with a drill bit diameter of 0.9 mm for $\delta^{15}\text{N}_{\text{AA}}$ analysis.

The coral skeletal samples were prepared for $\delta^{15}\text{N}_{\text{AA}}$ analysis after HCl hydrolysis and *N*-pivaloyl/isopropyl (Pv/iPr) derivatization, according to the procedure in (Chikaraishi et al. 2009, 2010). Briefly, the $\delta^{15}\text{N}$ composition of seven amino acids (Ala, Pro, Glu, Val, Leu, Phe, and Gly) was determined by gas chromatography/isotope ratio mass spectrometry (GC/IRMS) using a 6890N GC (Agilent Technologies) instrument coupled to a DELTAplus XP IRMS instrument through combustion (950°C) and reduction (550°C) furnaces, countercurrent dryer (Permeable membrane, NafionTM), and liquid nitrogen CO₂ trap via a GC-C/TC III interface (Thermo Fisher Scientific, Bremen, Germany). These amino acids were chosen because they have relatively constant recovery ($< \pm 10\%$) among replicate analyses in this method. The area data of each AA from GC/IRMS chromatograms was used to approximate quantification of AAs. To assess the reproducibility of the isotope measurement and to obtain the isotopic composition, reference mixtures of nine amino acids (alanine, glycine, leucine, norleucine, aspartic acid, methionine, glutamic acid, phenylalanine, and hydroxyproline) with known $\delta^{15}\text{N}$ values (ranging from -25.9% to $+45.6\%$), Indiana University, Bloomington, IN, SI science, Sugito-machi, Japan (Sato et al. 2014) were analyzed after every five to eight samples runs, and three pulses of reference N₂ gas were discharged at the beginning and end of each chromatography run for both reference mixtures and samples. The $\delta^{15}\text{N}$ composition of the coral skeletal amino acids was reported relative to air on scales normalized to known $\delta^{15}\text{N}$ values of the reference amino acids. The accuracy and precision for the reference mixtures were always 0.0‰ (mean of Δ) and 0.4–0.7‰ (mean of 1σ) for sample sizes of ≥ 1.0 nmol N, respectively. We report this analytical error (the accuracy and precision) because the numbers are well known based on the large number of runs of the reference mixtures. This precision is similar to that reported by recent studies (i.e., Sherwood et al. 2011; Bradley et al. 2015) for samples measured in triplicate ($< 1.0\%$). A sample chromatogram demonstrates the quality of the derivatization process of the coral skeleton (Supporting Information Fig. S1). The size of the drill bit used and the radial growth rate of the coral [~ 0.19 mm yr⁻¹ (Williams and Grotoli 2010a)] results in samples with a resolution of ~ 5 yr per measurements. A ¹⁴C-bombcurve-derived chronology for the same coral presented in Williams and Grotoli (2010a) served as a reference chronology to assign time to the $\delta^{15}\text{N}_{\text{AA}}$ measurements with an uncertainty of ± 2 yr.

To test for changes in the isotopic composition of the base nitrate to the food web, we measured the $\delta^{15}\text{N}$ of the

source amino acid Phe ($\delta^{15}\text{N}_{\text{Phe}}$). The $\delta^{15}\text{N}$ of Gly ($\delta^{15}\text{N}_{\text{Gly}}$) was also measured to test for its use as a source amino acid.

To test for potential microbial resynthesis of organic matter in the coral's food, we calculated the ΣV parameter as the average deviation in the $\delta^{15}\text{N}$ values of the Tr-AA Ala, Pro, Glu, Val, and Leu,

$$\Sigma\text{V} = 1/n\Sigma\text{Abs}(\chi_{\text{AA}}), \quad (1)$$

where χ (deviation) of each Tr-AA = [$\delta^{15}\text{N}_{\text{AA}}$ – average $\delta^{15}\text{N}$ (Ala, Pro, Glu, Val, and Leu)], and n = the total number of Tr-AA used in the calculation. The equation has been modified from McCarthy et al. (2007) to account for the trophic amino acids measured here.

To determine the trophic structure of the coral and its food source, we calculated the average $\delta^{15}\text{N}$ of the five trophic amino acids Ala, Pro, Glu, Val, and Leu ($\delta^{15}\text{N}_{\text{AA-Tr}}$). The uncertainty of the averaged $\delta^{15}\text{N}_{\text{AA-Tr}}$ value was determined using the standard error of the average of the individual $\delta^{15}\text{N}$ of the trophic amino acids. Following (Chikaraishi et al. 2009), we used the relationship between Glu and Phe to determine the trophic position of the DSC,

$$\text{TP} = ((\delta^{15}\text{N}_{\text{Glu}} - \delta^{15}\text{N}_{\text{Phe}}) - 3.4)/7.6 + 1, \quad (2)$$

where 3.4 is the β value of the difference between $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ in primary producers for aquatic cyanobacteria and algae and 7.6 is the average trophic enrichment factor (TDF) between Glu and Phe for each trophic position (Chikaraishi et al. 2009). The trophic position was calculated a second way making use of all of the measured trophic amino acids,

$$\text{TP} = (\delta^{15}\text{N}_{\text{ave AA-Tr}} - \delta^{15}\text{N}_{\text{Phe}}) - 2.4/6.38 + 1, \quad (3)$$

where 2.4 is the β value of the difference between the average $\delta^{15}\text{N}_{\text{AA-Tr}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ in primary producers (from Bradley et al. 2015 based on published values from Chikaraishi et al.; McClelland and Montoya 2002; McCarthy et al. 2013) and 6.38 was determined by calculating the average TDF values between each Tr-AA and Phe (from Bradley et al. 2015).

Results

The relative amount of each amino acid present in the coral skeleton varied (Fig. 2). Phe was present in the smallest amounts (average of 1% for all samples), followed by Pro (5%), Glu (5.2%), Leu (6.3%), Ala (9.0%), Val (9.6%), and Gly (46.5%).

The $\delta^{15}\text{N}_{\text{Phe}}$ composition (0.6‰ to 4.8‰) differed from that of the $\delta^{15}\text{N}_{\text{AA-Tr}}$ values, which themselves closely grouped together and all had overlapping ranges (total range 13.5‰ to 24.4‰) (Figs. 3a, 4b). On average, the $\delta^{15}\text{N}_{\text{AA-Tr}}$ values were isotopically more enriched than $\delta^{15}\text{N}_{\text{Phe}}$ by 14.2‰. The average $\delta^{15}\text{N}_{\text{Gly}}$ composition was between that of $\delta^{15}\text{N}_{\text{Phe}}$ and the average $\delta^{15}\text{N}_{\text{AA-Tr}}$ values (Figs. 3a, 4b). The

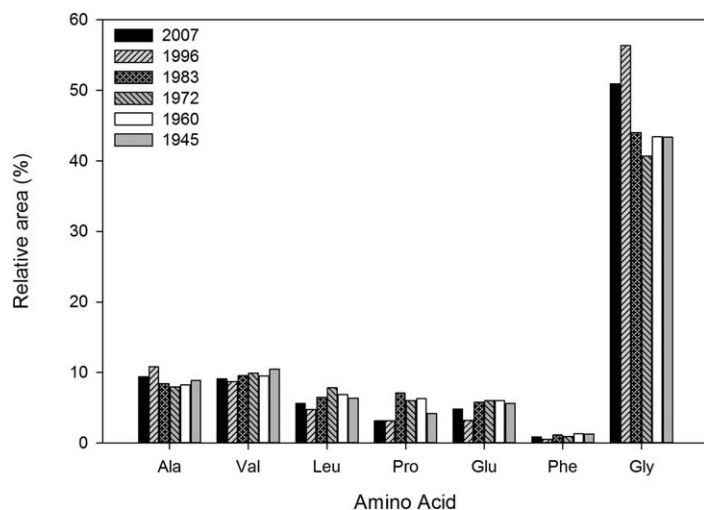


Fig. 2. The relative amount of each amino acid in the skeleton determined from the area data from the GC combustion IRMS chromatograms.

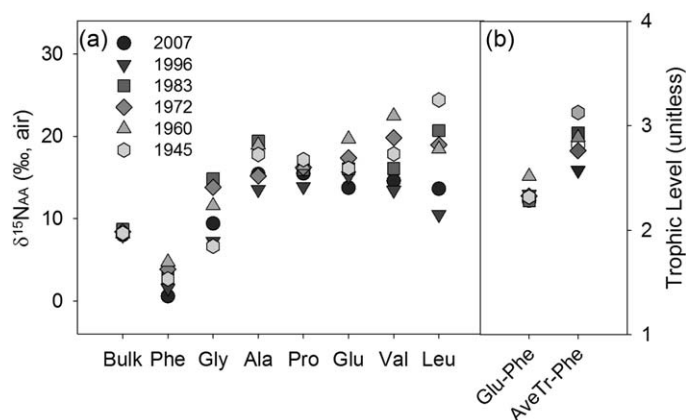


Fig. 3. (a) $\delta^{15}\text{N}$ values for six amino acids sampled at six time intervals over the coral's lifespan. Leu was not measured for the 1996 time interval. (b) Trophic position determined for the same six time intervals as (a) based on Eq. 1 in the main text. Uncertainty was less than size of the symbol for (a) and (b).

calculated average trophic position over the lifespan of the coral determined from Eq. 2 was 2.3 and determined from Eq. 3 was 2.8 (Figs. 3b, 4d).

The $\delta^{15}\text{N}$ values of the bulk coral skeleton and the average $\delta^{15}\text{N}_{\text{AA-Tr}}$ values significantly decreased from 1945 to 2005 (slope = -0.01 ± 0.001 , $p < 0.0001$ and slope = -0.086 ± 0.022 , $p = 0.018$, respectively) (Fig. 4a,b). In addition to the long-term trend, the average $\delta^{15}\text{N}_{\text{AA-Tr}}$ values declined from 19.2‰ to 14.0‰ from the late 1970s to the late 1990s, then increased to 14.6‰ to 2005 (Fig. 4b). The $\delta^{15}\text{N}_{\text{Phe}}$ value increased from 2.7‰ to 4.8‰ from 1945 to the early 1960s then significantly declined from 4.8‰ to 0.6‰ from the early 1960s to 2005 (slope = -0.093 ± 0.003 , $p < 0.0001$) (Fig. 4b). The $\delta^{15}\text{N}_{\text{Gly}}$ record did not significantly change linearly

over the duration of the record, instead increased from 6.7‰ to 14.8‰ from the start of the record to the mid-1980s, and then decreased to 7.3‰ followed by an increase to 9.4‰ (Fig. 4b). The ΣV parameter declined significantly over the record from 0.46 to 0.14 (slope = -0.004 , SE = 0.001, p -value = 0.040) when all trophic amino acids were included. However, the ΣV parameter did not significantly change over time if Leu was not included in the calculation of the ΣV parameter due to the one high $\delta^{15}\text{N}_{\text{Leu}}$ value in 1945 (slope = -0.002 , SE = 0.003, p -value = 0.53) (Fig. 4c). The trophic position of the coral was stable over the duration of the record when determined from Eq. 2 (slope = -0.002 , SE = 0.002, p -value = 0.33) and Eq. 3 (slope = -0.0065 , SE = 0.004, p -value = 0.15) (Fig. 4d).

Discussion

The calculated trophic position of the coral was 2.3 and 2.8 determined using the Glu-Phe Eq. 2 and the average Tr-AA – Phe Eq. 3, respectively (Fig. 3b). The coral will feed on food one trophic position below itself, which suggests a food source to the coral of trophic position 1.3. This trophic position is typical for particulate organic matter (McCarthy et al. 2007; Batista et al. 2014) and consistent with coral feeding on suspended particulate organic matter at the base of the euphotic zone (Williams and Grottoli 2010a,b). This trophic position calculated for the *Muricella* coral was higher than that calculated for deep-sea black corals from the Gulf of Mexico at 300-m depth of ~ 2 determined using Glu-Phe Eq. 2 (Prouty et al. 2014) but less than that reported for the gold coral *Kulamanamana haumea* specimens from the Island of O'ahu in the Hawaiian Islands at 450-m depth with an averaged calculated trophic position of 3.0 using Glu-Phe Eq. 2 and 2.7 using Eq. 3 with all available trophic and source amino acids (Sherwood et al. 2013). These deep-sea corals, located hundreds of meters deep in the water column, feed on sinking particulate organic matter exported out of the euphotic zone (Williams et al. 2007; Roark et al. 2009) in contrast to the *Muricella* coral analyzed here, located at the base of the euphotic zone with a diet of suspended particulate organic matter produced in situ (Williams and Grottoli 2010b). Thus, while azooxanthellate proteinaceous corals are deriving food from the particulate organic matter in the water column, there are notable differences in the form and composition of the specific food types feeding the corals depending on taxa and/or depth.

The stable trophic position of the *Muricella* coral over the duration of the entire record (Fig. 4d) suggests no change in autotrophic to heterotrophic ratio comprising the suspended particulate organic matter available to the coral for food. Thus, these data suggest that no substantial shift in primary productivity on decadal timescales occurred in the WPWP including no variability in response to the strong PDO shift in 1977. This is further supported by the general stability between the $\delta^{15}\text{N}_{\text{AA-Tr}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ offset (within analytical

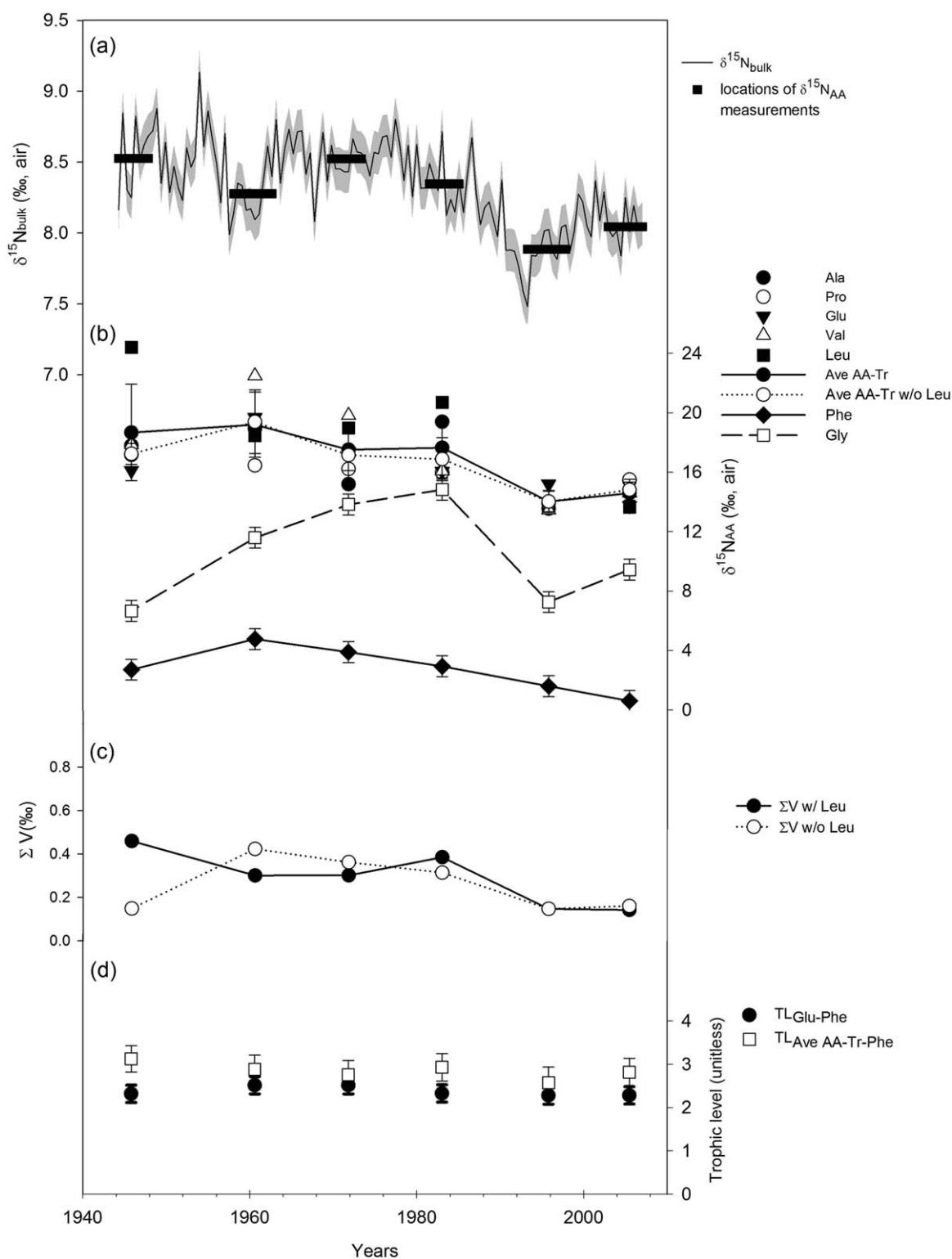


Fig. 4. (a) Bulk skeletal $\delta^{15}\text{N}$ composition for the same coral (data from Williams and Grottoli 2010a), (b) $\delta^{15}\text{N}$ of the trophic amino acids (AA) Val, Leu, Pro, and Glu, the source amino acid Phe, and Gly, and (c) ΣV parameter, a measure to test the extent of microbial resynthesis of organic matter determined using Eq. 2 in the main text, and (d) the calculated trophic position using Eq. 1 in the main text. The error bars for $\delta^{15}\text{N}_{\text{bulk}}$, $\delta^{15}\text{N}_{\text{Phe}}$, and $\delta^{15}\text{N}_{\text{Gly}}$ are instrumental error of $\pm 0.15\text{‰}$ for bulk values and $\pm 0.7\text{‰}$ for individual amino acids. The error bars for average $\delta^{15}\text{N}_{\text{AA-Tr}}$ are the standard error of the mean. The error bars in (c) are propagated error taking into account the 1σ error for the $\delta^{15}\text{N}_{\text{Glu}}$ and $\delta^{15}\text{N}_{\text{Phe}}$ measurements. AA = amino acid, Ave = average.

and statistical error, and particularly if the one high Leu value in 1945 is discarded) in the coral skeleton (Fig. 4b). In contrast, the small but significant decrease in the ΣV parameter indicates decreasing microbial resynthesis of organic matter that contributes to the corals food over its lifespan (Fig. 4c). The lack of a trend in the ΣV parameter without Leu, however, suggests that this result may be an artifact of the one high Leu value in 1945. Regardless, the low value of the ΣV parameter indicates overall minimal degradation of the organic matter and is a value consistent with that measured in algae from culturing experiments and the central Pacific Ocean (McCarthy et al. 2007). This supports a large contribution of fresh organic matter to the suspended POM feeding the *Muricella* coral and thus the coral is capturing in its skeleton in situ changes in the organic matter. In contrast, the average ΣV parameter of 3.14 determined for the Hawaiian *K. haumea* is indicative of sinking POM reaching the base of the mixed layer (McCarthy et al. 2007; Sherwood et al. 2013). These results further support that food sources to proteinaceous corals differs with taxa and/or depths.

The largest concentration of amino acid in the skeleton was Gly (Fig. 2), consistent with *K. haumea* coral but not the gorgonian coral *Primnoa resedaeformis* from the NW Atlantic Ocean, the latter of which had the highest molar concentrations of Ala followed by aspartic acid and then Gly (Sherwood et al. 2006, 2013). The $\delta^{15}\text{N}_{\text{gly}}$ composition of the *Muricella* coral here varied by more than double any of the other amino acids over the coral's lifespan, and largely explains the variability in the $\delta^{15}\text{N}_{\text{bulk}}$ values (Fig. 4a,b). While Gly has traditionally been classified as a source amino acid (Popp et al. 2007; Sherwood et al. 2011), Gly has multiple biosynthetic and metabolic pathways and the behavior of $\delta^{15}\text{N}_{\text{gly}}$ across trophic levels in food webs is complex (Chikaraishi et al. 2007). Recent studies indicate $\delta^{15}\text{N}_{\text{gly}}$ enrichment occurs with trophic transfer in organisms with substantial lack of dietary protein and thus $\delta^{15}\text{N}_{\text{gly}}$ may not reflect the $\delta^{15}\text{N}$ of the source nitrate (Chikaraishi et al. 2015; McMahon et al. 2015). The enriched $\delta^{15}\text{N}_{\text{gly}}$ composition relative to $\delta^{15}\text{N}_{\text{Phe}}$ measured in the coral is consistent with alteration of $\delta^{15}\text{N}_{\text{gly}}$ during trophic processing, as the $\delta^{15}\text{N}$ value of the Phe and Gly would be expected to be the same if they were both reflecting only the baseline $\delta^{15}\text{N}$ values. In the Hawaiian *K. haumea* coral and the NW Atlantic *P. resedaeformis* coral, $\delta^{15}\text{N}_{\text{gly}}$ is elevated relative to $\delta^{15}\text{N}_{\text{Phe}}$ yet still follows the same trend trends over the lifespan of the corals analyzed (Sherwood et al. 2011, 2013). In contrast in the deep-sea black corals, $\delta^{15}\text{N}_{\text{Phe}}$ and $\delta^{15}\text{N}_{\text{gly}}$ are the same (Prouty et al. 2014). Thus, the enrichment of $\delta^{15}\text{N}_{\text{gly}}$ relative to $\delta^{15}\text{N}_{\text{Phe}}$ may reflect either regional nitrogen dynamics or coral-taxa variability.

Comparisons of $\delta^{15}\text{N}_{\text{Phe}}$ with baseline $\delta^{15}\text{N}$ values in proteinaceous corals and other marine organisms support the use of $\delta^{15}\text{N}_{\text{Phe}}$ as a single source amino acid because its dom-

inant metabolic processes does not form or cleave bonds associated with the nitrogen atom (Chikaraishi et al. 2009, 2015; Sherwood et al. 2013; McMahon et al. 2015). In addition, the $\delta^{15}\text{N}_{\text{Phe}}$ composition measured in the coral (Figs. 3a, 4b) is consistent with low $\delta^{15}\text{N}$ of nitrate at the base of the euphotic zone in the WPWP (Yoshikawa et al. 2006). Thus here, we use the *Muricella* coral $\delta^{15}\text{N}_{\text{Phe}}$ composition to track changes in baseline $\delta^{15}\text{N}$ values at one site in the WPWP.

The maximum $\delta^{15}\text{N}_{\text{Phe}}$ value, and thus baseline $\delta^{15}\text{N}$ values, in the early 1960s followed by the decline to 2007 is intriguing. There are three potential mechanisms that could drive the variability in the baseline nitrate isotopic composition in the WPWP euphotic zone base: (1) changes in nitrate utilization, (2) changes in the source nitrate to the region, and (3) changes in atmospheric nitrogen to the WPWP. The shallow $0.1 \mu\text{mol kg}^{-1}$ nitricline in the WPWP means that low levels of nitrate are available at the base of the euphotic zone to support primary productivity (Yoshikawa et al. 2006) (Fig. 1). A shoaling of the $4 \mu\text{mol kg}^{-1}$ nitricline increases nitrate concentrations at the base of the euphotic zone. This decreases isotopic fractionation related to nitrate assimilation by primary producers because the relative nitrate utilization would be lower, and thus decreasing the $\delta^{15}\text{N}$ values of the organic matter produced in situ (Altabet and Francois 1994; Yoshikawa et al. 2006). Thus, a shoaling of the nitricline decreases skeletal $\delta^{15}\text{N}$ values and a deepening of the nitricline will increase skeletal $\delta^{15}\text{N}$ values. The measured decrease in coral $\delta^{15}\text{N}_{\text{bulk}}$ values and $\delta^{15}\text{N}_{\text{AA}}$ is consistent with the documented shoaling of the thermocline from the late 1970s to late 1990s and subsequent suggestion of an increase in $\delta^{15}\text{N}_{\text{bulk}}$ values and $\delta^{15}\text{N}_{\text{AA-Tr}}$ is consistent with the deepening of the thermocline post-late 1990s (Fig. 4a,b). However, the variability in $\delta^{15}\text{N}_{\text{Phe}}$ is not consistent with the thermocline/nitricline depth variability, indicating other influences on the $\delta^{15}\text{N}$ of nitrate at the base of the euphotic zone in the WPWP.

The relative contribution of the different water masses with unique $\delta^{15}\text{N}_{\text{NO}_3^-}$ values bathing Palau determines the source nitrate $\delta^{15}\text{N}$ in the region. The $\delta^{15}\text{N}_{\text{NO}_3^-}$ -enriched North Equatorial Current [$\delta^{15}\text{N}_{\text{NO}_3^-} = 7.5\text{‰} \pm 0.1$ at 7°N , 155°W , $n=2$; (Rafter et al. 2012)] feeds into the Mindanao Eddy, which in turn also supplies the nitrate-poor North Equatorial Counter Current (NECC), and bathes Palau (Heron et al. 2006). In contrast, nitrogen fixation in the far western equatorial Pacific supplies water with $\delta^{15}\text{N}_{\text{NO}_3^-}$ -depleted values (Yoshikawa et al. 2006). Thus, changing the ratio of the $\delta^{15}\text{N}_{\text{NO}_3^-}$ -enriched water from the North Equatorial Current relative to the $\delta^{15}\text{N}_{\text{NO}_3^-}$ -depleted water from the far western equatorial Pacific could potentially explain variability in the $\delta^{15}\text{N}$ values. In fact, an increase in eastern Pacific-El Niño intensifies and shifts NECC southward during the latter part of the calendar year which would drive variation in the $\delta^{15}\text{N}_{\text{NO}_3^-}$ reaching the euphotic zone around

Palau (Hsin and Qiu 2012). This would increase the contribution of Mindanao Eddy $\delta^{15}\text{N}$ -sourced nitrate with potentially higher $\delta^{15}\text{N}$. There were strong eastern Pacific El Niño events between the early 1980s and late 1990s (McPhaden et al. 2011), coinciding in timing with the declining $\delta^{15}\text{N}$ values. Therefore, attributing the change in *Muricella* coral $\delta^{15}\text{N}_{\text{bulk}}$ and $\delta^{15}\text{N}_{\text{AA}}$ skeletal values to shifts in the NECC is not consistent with the interannual drivers of variability in the NECC and changes in the source nitrate to the region also do not clearly explain the coral skeletal $\delta^{15}\text{N}_{\text{Phe}}$ variability.

A third explanation for the decreasing $\delta^{15}\text{N}_{\text{Phe}}$ is an increase in atmospheric nitrogen via nitrogen fixation to the WPWP. Sherwood et al. (2013) report an increase in nitrogen fixation starting in the mid-1800s, at the end of the Little Ice Age, in the subtropical North Pacific Ocean contributing low $\delta^{15}\text{N}$ to the base of the food web. The multidecadal variability found in the Palauan coral $\delta^{15}\text{N}_{\text{Phe}}$ may be part of this multicentennial variability. Additional older coral specimens from the WPWP are required to test if the trend measured here is part of longer term and broader pattern in environmental variability in the tropical Pacific Ocean.

Implication

Our results highlight the potential of compound specific stable isotope measurements in proteinaceous corals to unravel changes in nitrogen dynamics and primary productivity. While this is a relatively new technique, the consistency in the trophic position of the coral over time despite decadal timescale changes physical oceanography indicates that there is no resulting variability in primary productivity altering the trophic position of the gorgonian coral. Therefore, based on these data we highlight the need for a reappraisal of the importance of decadal nitricline variability when investigating carbon cycling around Palau in the WPWP, particularly in light of current external forcing of the Pacific Ocean.

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Conflict of Interest

None declared.

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