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Coral Reefs

Published: 12/04/2021

Peer reviewed version

Nitrogen enrichment in macroalgae following mass coral mortality

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Abstract

Scleractinian corals are engineers on coral reefs that provide both structural complexity as habitat and sustenance for other reef-associated organisms via the release of organic and inorganic matter. However, coral reefs are facing multiple pressures from climate change and other stressors, which can result in mass coral bleaching and mortality events. Mass mortality of corals results in enhanced release of organic matter, which can cause significant alterations to reef biochemical and recycling processes. There is little known about how long these nutrients are retained within the system, for instance within the tissues of other benthic organisms. We investigated changes in nitrogen isotopic signatures ($\delta^{15}N$) of macroalgal tissues a) ~1 year after a bleaching event in the Seychelles and b) ~3 months after the peak of

a bleaching event in Mo'orea, French Polynesia. In the Seychelles, there was a strong association between absolute loss in both total coral cover and branching coral cover and absolute increase in macroalgal $\delta^{15}N$ between 2014 and 2017 (adjusted $r^2 = 0.79$, $p = 0.004$ and adjusted $r^2 = 0.86$, $p = 0.002$, respectively). In Mo'orea, a short-term transplant experiment found a significant increase in $\delta^{15}N$ in *Sargassum mangarevense* after specimens were deployed on a reef with high coral mortality for ~3 weeks ($p < 0.05$). We suggest that coral-derived nutrients can be retained within reef nutrient cycles, and that this can affect other reef-associated organisms over both short- and long-term periods, especially opportunistic species such as macroalgae. These species could therefore proliferate on reefs that have experienced mass mortality events, because they have been provided with both space and nutrient subsidies by the death and decay of corals.

Key words: *Climate change; macroalgal bioindicators; coral bleaching; stable isotopes; biogeochemical cycles; coral reef ecology*

Introduction

Tropical coral reefs are highly productive ecosystems, but as they are typically surrounded by oligotrophic waters, they require constant recycling and retention of water-borne nutrients and organic matter (Galloway et al., 2004). There are a wide range of physical and biological processes on coral reefs which can retain these essential energetic resources within local biogeochemical cycles for extended periods of time. Thus, these processes can sustain rapid rates of biological activity such as primary productivity, as well as many other key ecosystem functions (Wyatt et al., 2013). For instance, coral-derived particulate organic matter (POM) in the form of mucus can act as an energy carrier and particle trap, so these nutrients may be

recycled by benthic and planktonic communities over longer timescales (Ferrier-Pagès et al., 1998; Wild et al., 2004a,b). However, even in a coral-dominated ecosystem, they are not the only natural, or autochthonous, source of bioavailable nutrients (Davey et al., 2008; Wyatt et al., 2013). Microbes, for instance, are capable of nitrogen fixation (Moulton et al., 2016), and other primary producers, such as phytoplankton and macroalgae, readily take up and store nutrients and dissolved organic matter (DOM) in their tissues (Fong et al., 1994). This DOM is then recycled either through tissue breakdown or through consumption by higher trophic level organisms such as herbivorous fishes, which in turn recycle significant amounts of nutrients through excretion (Burkepile et al., 2013).

Healthy coral reefs typically persist in suboptimal nutrient concentrations, although nutrient pulses can disrupt the balance of natural biogeochemical dynamics jeopardising reef health. Disturbances such as marine heat waves that cause coral bleaching have a direct negative impact on corals, but can also have indirect consequences for reefs by altering nutrient dynamics (D'Angelo & Wiedenmann, 2014). Branching scleractinian corals are often dominant on a reef, providing structural complexity and micro-habitats for a variety of reef-associated organisms, but they are also particularly vulnerable to heat stress (Hughes et al., 2019). The loss of these vital foundation species therefore has huge implications for the entire ecosystem (Graham et al., 2015; Wilson et al., 2019). Where coral bleaching causes extensive mortality, the metabolic exchange between corals and associated organisms on a reef is reduced, along with the capacity of corals to trap organic matter. This can subsequently trigger the dysfunction of major biogeochemical processes (Glynn, 1993; Wild et al., 2011).

There are few studies assessing how climate-derived disturbances affect mucus release by live corals, and associated processes. Davey et al. (2008) found that in the weeks that follow

coral bleaching, a 30-fold higher production of new nitrogen occurred on coral reefs compared to those that did not experience bleaching. Such nitrogen productivity has also been shown in an experimental setting (Niggl et al., 2009). While release rates of mucus-derived POM from corals increase during the early stages of bleaching, providing a burst of nutrients to coral reefs (Coffroth, 1990), these rates can decrease after the initial bleaching response (Fitt et al., 2009; Wooldridge et al., 2009). If corals recover from bleaching, which can take many weeks to occur (Gates, 1990), there may only be short- to medium-term effects on biogeochemical processes. However, if corals die, the subsequent mass release of coral tissue into reef environments may also alter biogeochemical processes, and over longer time scales. In addition, colonisation of the exposed coral skeleton by microbial biofilms, turf algae, macroalgae, sponges, cyanobacteria or other invertebrates may not only reduce coral recruitment success, but can also change biogeochemical processes such as nitrogen fixation (Diaz-Pulido & McCook 2002; Davey et al. 2008; Haas et al., 2010).

In order to identify changes in nutrient regimes due to mass coral mortality, nitrogen stable isotopes ($\delta^{15}N$) and nitrogen content (%N) can be analysed from macroalgal tissues to capture temporally-extensive records of nutrient loads (Costanzo et al., 2001). Stable isotopes of nitrogen have been used in nutrient studies for several decades, helping to identify the origins of nitrogen (Heaton, 1986; Kolasinski et al., 2011). In addition, certain types of marine algae are commonly used in biomonitoring studies due to their widespread distribution and responsiveness to bioavailable pollutants. *Sargassum*, for example, is a genus used worldwide as it has been found to be responsive to nutrient enrichment (Schaffelke & Klumpp, 1998; Schaffelke, 2002; García-Seoane et al., 2018). However, marine algae are not the only functional group that can be used to measure isotopic signatures as a proxy of nutrient regimes on reefs. Organisms at higher trophic levels also assimilate nutrients from lower

trophic levels, resulting in increasing isotopic enrichment up the food chain (Bierwagen et al., 2018). For instance, corals are at a higher trophic level than primary producers such as macroalgae, and thus have enriched isotopic signatures (Graham et al., 2018). As corals release organic matter into the water column after the death and subsequent decay of tissue following marine heatwave-driven mortality events (Leggat et al., 2019), opportunistic benthic species such as macroalgae may capitalise on this new nutrient source, assimilate it into tissues for growth and storage, and consequently become more enriched (Pawlik et al., 2016).

In the current study, the temporal effect of coral mass mortality on macroalgal stable isotopic signatures is investigated in two different coral reef systems, over two different time periods. As such, it offers new understanding on whether macroalgae can indicate longer-term effects of coral mortality events on reef nutrient dynamics and biogeochemical cycles. Specifically this study assesses: (1) changes in *Sargassum* sp. nutrient signatures over three years in the inner Seychelles Islands, western Indian Ocean, spanning a mass coral bleaching event, and (2) shorter-term changes in *Sargassum mangarevense* nutrient signatures ~3 months after the peak of a severe bleaching event in Mo'orea, French Polynesia, using an *in-situ* three-week transplant experiment.

Methods

Study Site 1: Seychelles

The inner Seychelles islands experienced two severe coral bleaching events, in 1998 and 2016. In 1998, coral cover dropped by 90%, and though hard coral cover steadily recovered on some study sites (average coral cover of 27% by 2014) (Graham et al., 2015), another global bleaching event in 2016 (Hughes et al., 2018) led to live coral cover declining by 70% on these same sites (Wilson et al., 2019). Around the Inner Seychelles, heat stress reached 4°C-weeks in January 2016, rapidly increased in April and peaked at 11.4°C-weeks in May (Wilson et al., 2019; http://coralreefwatch.noaa.gov/vs/index.php).

Eighteen reefs were surveyed in April 2014, before the mass bleaching event caused extensive coral mortality in 2016 and again in April 2017, a year after the event occurred (Wilson et al., 2019). These reefs form part of a 25-year coral reef monitoring survey around the inner Seychelles, with roughly half the reefs having been defined as "recovering" from a previous mass bleaching event in 1998, and the other half as transitioning to a "regime-shifted" macroalgae-dominated state (Graham et al. 2015). Eight replicate 7-m radius point counts were surveyed along the reef slope on each reef for both survey years. Within each point count area, the percent cover of benthic categories including live hard coral, soft coral, macroalgae, sand, rubble, and rock was quantified using 10m long line-intercept transects (Wilson et al. 2019).

The objectives of this component of the study were to assess the relationship between changes in percent cover of corals between the study years of 2014 and 2017 with differences

148 in $\delta^{15}N$ and %N signatures in tissues of *Sargassum* sp. that were collected from the same sites
149 during the same surveys. Low availability of macroalgae at some reefs meant that macroalgae
150 for stable isotope analyses were not collected from all reefs in both years. A minimum of four
151 replicate *Sargassum* sp. samples were collected from each of the seven "coral mortality"
152 reefs (a subset of the previously termed "recovery reefs", named as such following the
153 impacts of the 2016 bleaching event) and from the six "regime-shifted" reefs in both 2014
154 and 2017.
155
156 **Study Site 2: Mo'orea**
157
158 Mo'orea, an island which is part of the Society Archipelago in French Polynesia, has
159 demonstrated rapid coral recovery from previous disturbances (Vercelloni et al., 2019;
160 Hédouin et al., 2020). For example, following an outbreak of *Acanthaster* spp. from 2006 to
161 2009 and a cyclone in 2010, mean coral cover on the outer reefs was reduced to 2% at 10 m
162 depth from a high of 39% in 2005, before recovering to 27% in just four years. The branching
163 coral genus *Pocillopora* spp. was found to be a significant driver in that recovery, as it made
164 up 53% of the re-established coral community (18% cover) (Tsounis & Edmunds, 2016).
165 There were no recorded episodes of abnormally high sea surface temperature (SST) in 1998
166 in Mo'orea, but it was impacted by the global coral bleaching event in 2016, with heat-
167 sensitive branching corals being the worst affected (Hughes et al., 2019). Donovan et al.
168 (2020) reported that 37% of *Acropora* and 28% of *Pocillopora* colonies exhibited bleaching
169 across all sites, with up to 100% bleaching of *Acropora* on north shore sites. Coral mortality
170 was rare (~1%), as heat stress did not exceed 1.1°C weeks (Hédouin et al., 2020).
171

Annual surveys of 13 marine areas around Mo'orea were established in 2004 (Service National d'Observation CORAIL). For the purpose of this study, data for the reef slope at the four areas along the north coast of the island, where bleaching was highest and our study site was located, was used (*Suppl. Fig. 1*). This includes the site Tiahura which is closest to our study site. The benthic cover of each sample area was quantified at a similar depth to the transplant site (~10 m) using 3 replicate non-permanent 25 m transects (Horta e Costa et al., 2016). The percentage cover of benthic components was sampled every 50 cm using the Point Intercept Transect (PIT) method. Macroalgae was categorised as all the non-coralline algae of large enough size to identify with the naked eye.

Sea surface temperature (SST) was measured hourly using an SBE-56 sensor (Sea Bird Scientific) on the Tiahura forereef at 3m depth from 1998 to 2005. The time series was interrupted for 5 years before being collected continuously again from 2010. In order to characterise the temperature trend in 2019, relative to that of other years, we calculated weekly means for 2019 and compared this with the average temperature time series and 95% confidence intervals for the entire period. In addition, following Donovan et al. (2020), we calculated cumulative heat stress (in °C weeks) as a 12-wk running sum for all temperatures exceeding 29 °C, a threshold that is considered a good predictor of bleaching in Mo'orea based on previous studies (Pratchett et al., 2013; Donovan et al., 2020; Hédouin et al., 2020). The maximum water temperature during 2019 exceeded 29 °C in March and peaked at ~30°C in April. Patterns of cumulative heat stress peaked at ~6 °C weeks. As the duration of heat stress was much longer in 2019 than in the previous bleaching event (Donovan et al., 2020; Hédouin et al., 2020), the extent of coral mortality was much higher (*Suppl. Fig.2*).

196 Samples of *Sargassum mangarevense* (n=10) were collected from Papetoai lagoon, a low-
197 nutrient reef in the northwest region of Mo'orea on 6th July 2019 (*Suppl. Fig.1*). These
198 waters were found to typically have low $\delta^{15}N$ and %N values, shown in nutrient heat maps in
199 Leichter et al. (2013) and Donovan et al. (2020). Specimens were placed in shaded coolers
200 filled with seawater before they were transported back to the CRIOBE research station,
201 Mo'orea. After all visible, larger epiphytes were carefully removed from the fronds using a
202 scalpel, initial tissue samples were taken and frozen at -20°C for later stable isotopic
203 analyses. Algal specimens were then placed in pre-transplant holding tanks for seven days,
204 with water changes every two days. Water changes in the tanks involved surface water
205 collected from the forereef, as it was found to typically be low in $\delta^{15}N$ (< 3.0 ‰, Lin & Fong,
206 2008, Donovan et al., 2020). This was done to ensure that internal nutrient stores in *S.*
207 *mangarevense* were depleted before specimens were transplanted on the forereef where there
208 were high levels of coral mortality. Following this seven-day acclimation period, further
209 tissue samples were taken for stable isotopic analyses. For the *in situ* macroalgal bioassay, a
210 cage was made out of chicken-wire mesh and attached to a cinder block that was already
211 placed on the forereef at ~12 m depth. At the time of the transplant experiment in July 2019,
212 while some corals were still bleached, ~40% had already died (S.J.H., 2020, *pers. obs.*). It
213 was not possible to have a control bioassay, due to restrictions on deploying additional cinder
214 blocks and the lack of non-bleached reefs at that time. The ten macroalgal specimens were
215 deployed on the reef for ~3 weeks from 15th July to 4th August 2019 before they were
216 collected and returned to CRIOBE. Final tissue samples were taken and frozen before stable
217 isotopic analyses were performed.
218
219
220 **Stable Isotopic Analyses**

All frozen samples from both studies were individually defrosted, rinsed thoroughly with fresh or distilled water, and placed in a drying oven for 48 h at 60°C. Once dried, samples were each ground into a fine powder using a ball mill and stored in individual airtight containers. All dried samples were weighed, alongside the relevant standards, for stable isotopic analyses. Samples were then run on an IsoPrime Dual Analyser to determine signatures of stable isotopes and elemental content. The stable isotopic ($\delta^{15}N$) and elemental analyses (%N) for both the 2017 samples from the Seychelles study and the 2019 Mo'orea samples were run on an Isoprime100 Isotope Ratio Mass Spectrometer (IRMS) linked to an Elementar VARIO MICROcube Elemental Analyser at Lancaster Environment Centre, Lancaster University. The samples collected in 2014 from the Seychelles were analysed using a Costech Elemental Analyzer fitted with a zero-blank auto-sampler at James Cook University's Advanced Analytical Centre, Cairns. Analyses from both years were standardised using internal reference materials calibrated to international standards.

Statistical Analyses

For the Seychelles data, four separate two-way analysis of variance (ANOVAs) were used to assess the effect of time period (two levels: 2014 and 2017), reef state (two levels: coral mortality and regime shift) and their interaction on a) total coral cover, b) branching coral cover, c), $\delta^{15}N$, and d) %N across all 13 reefs where *Sargassum* were consistently collected. Based on this analysis and subsequent post-hoc Tukey tests, we found that predominant changes in these response variables were observed on "coral mortality" reefs, with little response on "regime-shifted" reefs. We therefore include the seven reefs with high levels of coral mortality to investigate the relationship between changes in nutrient signatures against

a) absolute and b) branching coral cover loss, using linear regression models. This decision was further supported by coral cover changes on "regime-shifted" reefs, where starting absolute values in 2014 were already very low at 6.69 ± 1.8 % before dropping by ~5% in 2017, and macroalgal cover was very high in both years. Therefore, any influence of coral cover on nutrient signatures in the system would be negligible (*Suppl. Fig. 3*; Wilson et al., 2019).

For the Mo'orea data, differences between a) average $\delta^{15}N$ and b) %N signatures in the *Sargassum* specimens in the three treatments (initial, pre-transplanted, and post-transplanted) from the transplant experiment were analysed using a repeated measures ANOVA. Repeated measures were incorporated into this ANOVA as tissue samples were taken from the same experimental specimens placed under the three different treatments. A time series analysis was conducted to compare the average mean monthly SST in 2019, relative to SST in previous years. Normality of data was assessed visually, and homogeneity of variance for all ANOVAs conducted for both studies was assumed with a Levene's test. All statistical analyses were conducted in R (R-Core-Team 2018), and the time series analyses for Moorea were performed using 'zoo' and 'xts' packages to produce *Supplementary Figure 2* (Zeileis & Grothendieck. 2005; Ryan & Ulrich, 2020).

Results

Seychelles

There was a significant effect of year, reef state and interaction on total coral cover across the thirteen reefs (interaction: $F_{1,204} = 37.3$, $p < 0.0001$). The post-hoc Tukey test revealed that there was no significant difference between the pre- and post-bleaching years for the "regime-shifted" reefs ($p = 0.32$; *Suppl. Fig. 2*). In contrast, the seven "coral mortality" reefs declined significantly from 27.0 ± 1.5 to 8.01 ± 0.5 % between 2014 and 2017 ($p < 0.0001$; *Fig. 1a*). This was mainly due to a loss in branching coral cover on these reefs from 16.0 ± 1.5 to 0.30 ± 0.05 % ($p < 0.0001$; *Fig. 1a*). Percent cover of massive corals remained similar between 2014 and 2017 on "coral mortality" reefs, whereas table coral cover declined from 1.27% to 0%. There was also a 0.8 % increase in total macroalgal cover on the seven study reefs between the years.

The $\delta^{15}N$ signature in *Sargassum* tissues differed significantly between 2014 and 2017 across all thirteen reefs (interaction between year and reef state, $F_{1,124} = 11.4$, $p = 0.001$), but only showed a significant difference for the seven "coral mortality" reefs between survey years ($p < 0.0001$, *Fig. 1b*; $p = 0.15$ for regime-shifted reefs). Similarly, %N in *Sargassum* tissues was higher in samples collected from "coral morality" reefs in 2017 than in 2014 ($p < 0.0001$, *Fig 1c*; significant interaction between year and state $F_{1,124} = 5.0$, $p = 0.03$), although there was no temporal difference in N content in samples collected from "regime-shifted" reefs ($p = 0.20$). For the seven "coral mortality" reefs selected for the purpose of this study, there was a significant positive relationship between increase in $\delta^{15}N$ in *Sargassum* tissue and (a) loss

of total coral (adjusted r^2= 0.79; p = 0.004; *Fig. 2*) and (b) branching coral cover (adjusted r^2 = 0.86; p = 0.002). There was no significant relationship between changes in %N and total coral cover (r^2 = 0.04; p =0.67) or branching coral cover (r^2 = 0.04; p = 0.66).

Moorea

Before the bleaching event peaked in April 2019 (*Suppl. Fig. 2*), the benthic cover survey conducted across the outer slopes of the four northern sites of Moorea in March 2019 showed an average of 73.7 ± 2.8% live coral cover, with a significant decline to an average of 36.2 ± 2.9 % in 2020, a year after the event (p < 0.0001; Mean ± SE). The closest site to the transplant experiment, Tiahura, had 73.3 ± 5.5% and 36.0 ± 2.0% in live coral cover in 2019 and 2020, respectively. The high coral cover across the four sites in 2019 was primarily due to the abundance of branching coral *Pocillopora* on the forereefs in Mo'orea (Tsounis & Edmunds, 2016). For instance, at Tiahura, there was 60.7 ± 5.7% cover of *Pocillopora* and an average of 55.5 ± 3.3% cover across the four sites in 2019. When the survey was repeated in March 2020, there was a significant decrease in *Pocillopora* to 24.5 ± 1.7% across all four sites (p < 0.0001), and a similar pattern was shown at Tiahura (p < 0.0001). Other than this predominant branching coral, no significant differences were found between the years for the other reef-associated organisms, including other coral genera.

In the short-term transplant experiment shortly after the peak of the bleaching event in Mo'orea, treatment had a significant effect on macroalgal $\delta^{15}N$ signatures (repeated-measures ANOVA: $F_{2,27}$ = 31.71, p <0.0001; *Fig. 3*). Post hoc tests indicated that there were significant differences in $\delta^{15}N$ between all three treatments (initial, pre-transplant, and post-transplant, n=10), which suggested that $\delta^{15}N$ declined in the pre-transplant holding tanks, and then

increased substantially on the transplant reef (initial and pre-transplant: $p = 0.003$; initial and post-transplant: $p < 0.0001$; pre-transplant and post-transplant: $p < 0.0001$). However, there was no significant effect of treatment on macroalgal %N (repeated-measures ANOVA: $F_{2,23} = 0.6$, $p = 0.58$; *Suppl. Fig. 4*). Although it was not possible to include either control sites or reefs with varying levels of bleaching due to permit restrictions, the benthic data shows that the extent of mortality across the outer slopes on the northern region of Mo'orea was quite similar.

Discussion

The current study suggests that mass coral mortality events can be detected through nitrogen isotopic signatures in macroalgal tissues, a proxy for nutrient sources, up to a year after a severe bleaching event. Although the exact source of the enrichment could not be traced in this study, a significant increase in $\delta^{15}N$ was shown in both reef systems over different timescales after two separate coral mortality events. For instance, in the Seychelles, there was a strong positive correlation between a decline in total coral cover and an increase in $\delta^{15}N$. This suggested that the N present in algal tissues could be coral-derived. These findings may help improve understanding of how mass disturbances such as coral bleaching impact multiple ecosystem processes on climate-impacted reefs. For instance, the loss of live coral cover, especially branching corals, provides a large amount of new substrate for opportunistic species such as macroalgae and other primary producers to colonise and prevent coral recovery, and may also provide an additional source of nutrients which become locked in the system. Consequently, this could enhance macroalgal proliferation on this colonised space, reinforcing alternative regimes.

The isotopic signature of fleshy macroalgae changed significantly over both short and long timeframes following bleaching events on two different reef systems. The positive relationship between $\delta^{15}N$ and the declines in coral cover suggest that nutrients from dead and decaying corals have contributed to this change of isotopic signatures in macroalgae. While this might be an important natural source of nutrients (Coffroth, 1990; Brown & Bythell, 2005; Bythell & Wild, 2011), any substantial increase could affect or disrupt natural metabolic exchanges between corals and other organisms, not only with their endosymbiotic zooxanthellae, but with sponge, seaweed and microbial communities (de Goeij et al., 2013;

Rix et al., 2016, 2017; Pawlik et al., 2016; Mumby & Steneck, 2018; Leggat et al., 2019). Much of the literature focuses on the mucus released from live corals and how it is recycled within the system (Davey et al., 2008; Naumann et al., 2009; Wild et al., 2004a,b, 2010, 2011), as well as the short term effects of changes in organic matter release after a bleaching event (Niggl et al., 2009; Wooldridge, 2009). Other work such as Radice et al., (2020) supports this by showing that isotopic signatures of particulate organic nitrogen in the water column decreased eight months after a bleaching event. However, there is still little understanding of changes in reef biogeochemical cycles.

Excess nutrients are one of the key factors that can drive a bleached reef towards a regime shift (Graham et al 2015). If the increased release of organic matter through mass coral mortality provides more nutrients to opportunistic species, this may encourage fast-growing macroalgae to proliferate on the exposed coral skeletons. This negative feedback loop can inhibit coral recovery and foster regime shifts to macroalgal-dominated states (Diaz-Pulido & McCook, 2002; Haas et al., 2010; Wild et al., 2011). For instance, the lack of available substrata may reduce the ability for any coral larvae to colonise this space and repopulate reefs, but increases in algal-derived DOM and POM can subsequently increase pathogenic microbial activity through what has been termed the DDAM positive feedback loop (dissolved organic carbon, disease, algae, microorganisms) (Haas et al., 2016). Macroalgae release labile organic matter which benefit pathogenic microbes and together they create unfavourable conditions for corals. For example, they collectively disrupt the function of the coral holobiont, thereby exacerbating death of coral recruits, and maintaining competitive dominance in algae (Wild et al., 2010; Barott & Rohwer, 2012; Pawlik et al., 2016; Mumby & Steneck, 2018).

While mass mortality has the potential to release a substantial source of new nutrients, this type of organic matter is still considered to be internal, or autochthonous (Briand et al., 2015). Excessive nutrient enrichment from external anthropogenic nutrient loads, particularly certain types of nitrogen such as nitrates found in coastal runoff, can further exacerbate changes in biogeochemical cycles on reefs (Burkepile et al., 2020; Donovan et al., 2020). This could accelerate the proliferation of macroalgae and other opportunistic organisms, and further decrease the chance of scleractinian corals re-establishing themselves. In addition, declines in water quality can develop and cause the formation of algal blooms (Fabricius, 2005; Tanaka et al., 2010).

Fleshy macroalgae are important indicators of changes in nutrient cycles because the bioavailable nutrients which are taken up from the water column and assimilated into their tissues can be easily measured over both short and long periods of time (Costanzo et al., 2001). Macroalgae have been used as proxies to study the effects of nutrient enrichment in both laboratory and *in situ* experiments, but these mostly tend to be for investigating anthropogenic sources, such as from coastal run-off (Fong et al., 1994; García-Seoane et al., 2018; Burkepile et al., 2020) and less commonly for natural nutrient inputs, such as seabird guano, deep-water upwelling events or coral-derived organic matter (Schaffelke, 2002; Graham et al. 2018; Williams et al., 2018).

The kind of nutrient signature used as a bioindicator is also an important factor to consider. Lin & Fong (2008) found $\delta^{15}N$ to be a more sensitive indicator to changes in nutrients in transplanted macroalgae than %N. Nitrogen content is typically diluted during rapid growth of specimens, suggesting that nutrients are only stored in macroalgal tissues over the long term when nutrient supply exceeds growth rate, as they first must assimilate excess nitrogen

into growth. This likely explains why we found no patterns in %N in neither the Seychelles regression analysis, nor the Mo'orea transplant experiment.

Although the duration of transplant experiments in the literature vary considerably, from hours to ~1 year, García-Seoane et al. (2018) recommended an exposure time of < 1 month, as the uptake kinetics of algal transplants can vary based on the species used or local environmental conditions. The current study suggests that these changes in nutrients may be detected in *Sargassum* tissues up to 12 months after an event, implying that nutrients have been trapped and retained in the system for at least a year. It is also known that *Sargassum* undergoes major seasonal fluctuations in production and biomass that may supplement adjoining ecosystems within the broader seascape (Fulton et al., 2019). This study supports previous literature suggesting macroalgae can easily be deployed in target areas to investigate changes in nutrient loads (Costanzo et al., 2001; García-Seoane et al., 2018), but also applies this common technique to capturing energetic resources. Therefore, macroalgal assays have the potential to provide insight into changes in nutrient sources from both natural and anthropogenic events, such as widespread coral bleaching.

There are a number of potential sources of nitrogen that could have influenced our results other than coral-derived nutrients. A strong nutrient gradient from the land-end of Opunohu Bay in Mo'orea to its ocean-end (Lin & Fong, 2008) suggests that the nutrient enrichment from the shrimp farm effluent entering the bottom of the bay was unlikely to affect the isotopic signatures of our specimens. However, storms and heavy rainfall can influence both the spatial extent of run-off and nutrient uptake in reef macroalgae (Clausing & Fong, 2016; Adam et al., 2020). Local upwelling could have provided nutrients and influenced our results, but Lin & Fong (2008) suggest that the $\delta^{15}N$ of tropical ocean seawater is typically ~3 ‰,

445 which is lower than the signatures found in both the post-transplant and pre-transplant tissue
446 samples. In addition, no *Sargassum* specimens were found at the depth where the bleaching
447 occurred in Mo'orea (~12m), so samples had to be taken from the nearby nutrient-limited
448 lagoon (~1 m). Although this lagoon typically has low nutrient levels (Donovan et al., 2020)
449 and the algal specimens collected from there had low tissue nutrient history, some bleaching
450 was observed in the lagoon at the time of collection, but not in the specific area where the
451 specimens were collected. Even if some coral-derived nutrients were captured by the initial
452 specimens, we accounted for this by depleting tissue nutrient stores in the holding tanks. This
453 resulted in a significant decline in $\delta^{15}N$, followed by a significantly higher signature in the
454 post-treatment algae after they were transplanted at the site where extensive coral bleaching
455 and mortality had occurred. Other factors such as light intensity can also affect algal
456 condition and isotopic signatures (Marconi et al., 2011; García-Seoane et al., 2018), so may
457 have also influenced results in Mo'orea.
458
459 Future research could build on this study, and on other studies in the literature (García-
460 Seoane et al., 2018) by applying the above methods to test the degree of influence of coral-
461 derived organic matter on macroalgal nutrient signatures, relative to anthropogenic sources,
462 either in laboratory- or field-based experiments. For instance, macroalgal bioassays could be
463 deployed on bleached reefs with low levels of coastal run-off, such as those in other regions
464 around Mo'orea, and compared to those with significantly higher levels, to test if these
465 effects are synergistic. Clearly assessment of macroalgal isotope signatures across different
466 nutrient loads and levels of coral mortality are required to fully understand nutrient sources
467 before attribution of nitrogen enrichment in macroalgae to nutrients released from dead and
468 decaying corals can be definitively determined.
469

While this study compared the $\delta^{15}N$ signatures in tissues of *Sargassum* from pre- and post-bleaching years in the Seychelles, no macroalgal samples were collected during the bleaching and the subsequent mortality event in 2016 itself, so it was not possible to compare the stable isotopic results when this mass tissue release was occurring. The short-term experiment in Mo'orea was conducted in part to understand these shorter-term dynamics and to further support these findings. Though the results from the two different reef systems are not directly comparable, this study suggests that macroalgal tissue $\delta^{15}N$ signatures can be affected by mass morality events. However, as the current study only implies that the mass release of dead coral tissue enriched the macroalgal $\delta^{15}N$ signatures, future research could expand on this work by determining the exact source(s) of enrichment (Briand et al., 2015). For instance, enriched stable isotope tracers (^{15}N and ^{13}C) (Naumann et al., 2010) or compound-specific stable isotopes (McMahon et al., 2016) could be used to quantify the flow of organic matter from dead corals to macroalgae in an experimental setting, or seawater from reefs with varying levels of coral mortality could be collected and used to test the responses of macroalgae.

In conclusion, this study highlights how mass coral mortality events, trigged by marine heat waves, may add additional sources of nutrients into coral reef biogeochemical cycles, which are available to opportunistic macroalgae. These changes in nutrient dynamics could have significant impacts on coral reefs, particularly if those sources are specifically becoming more available because key ecosystem engineers such as scleractinian corals are in decline (Wild et al., 2011). It also suggests that these nutrients can be retained within reefs and can have both short-term and long-term impacts on their biogeochemical cycles. Although it is not yet known how long these nutrients remain in the system, if other environmental conditions are favourable enough, then corals might still be able to recover (Graham et al.,

2015). However, if these same reefs are also facing other local anthropogenic stressors, such as nutrient runoff or overfishing of herbivores, then large coral mortality events may result in competitive advantages to benthic organisms such as macroalgae, leading to a benthic regime shift (Ainsworth et al., 2020). This emphasises the critical need to manage local stressors by detecting and reducing nutrient runoff and other drivers, especially on reefs that do still have high abundance of corals, and/ or have recently bleached.

Conflict of Interest Statement: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Acknowledgements

We thank Seychelles Fishing Authority, Seychelles Marine Parks Authority and Jan Dajka for field and laboratory assistance in the Seychelles, Centre de Recherches Insulaires et Observatoire de l'Environnement (CRIOBE) and Service National d'Observation CORAIL data and assistance in Mo'orea, and David Hughes and Jon Crosse from Lancaster Environment Centre for extensive help with preparations for fieldwork and with the subsequent stable isotopic and elemental analyses. The project was funded via a grant from the Royal Society (RG150710).

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Figure Legends

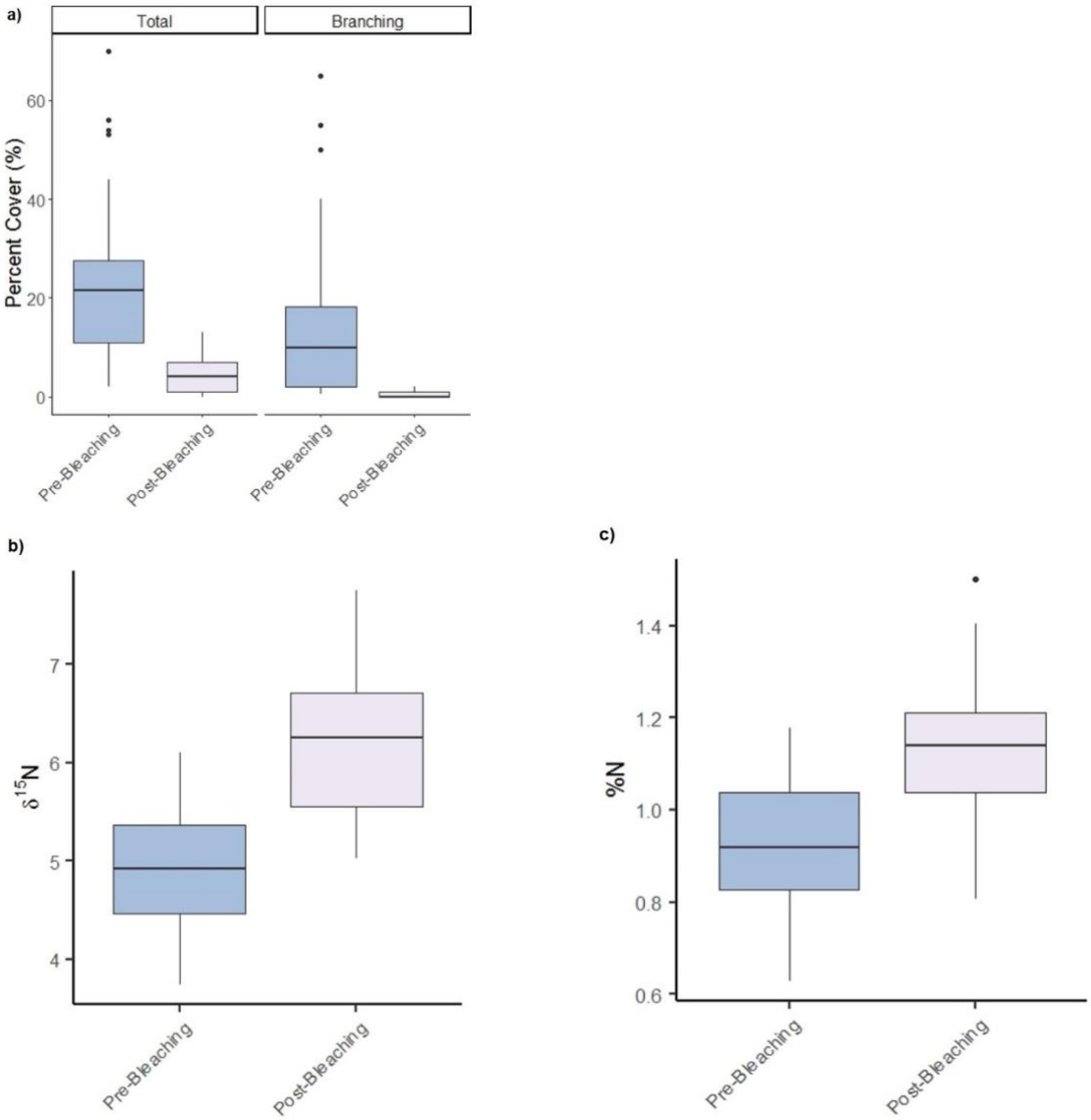

Figure 1. Box and whisker plots of the median a) total and branching coral cover in both pre-bleaching and post-bleaching years (2014 and 2017, respectively) on "coral mortality" reefs (n=7), b) the average $\delta^{15}N$ signatures in *Sargassum* sp. tissues in both years, and c) the average percent N (%N) in both years,. The pale blue boxes represent the pre-bleaching year and pale pink boxes represent the post-bleaching year, both showing the third quartile (Q3) and first quartile (Q1) range of the data, the whiskers (95% quartile) and data outliers.

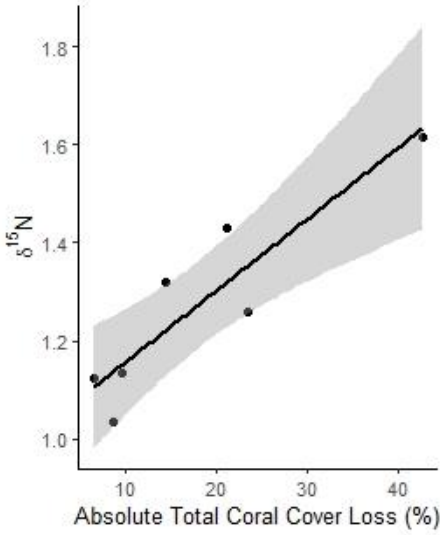

Figure 2. Change in absolute total coral cover and the corresponding changes in $\delta^{15}N$ in *Sargassum* tissues across seven coral mortality reefs in the Seychelles between 2014 and 2017. The regression lines and confidence intervals were obtained using linear regression coefficient of determination (r^2); 95% confidence intervals.

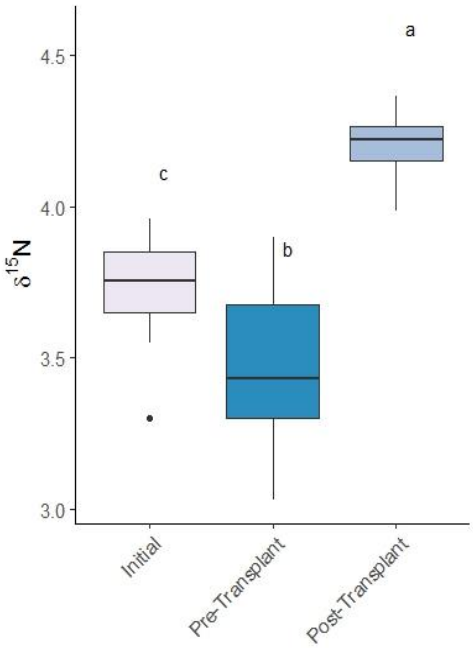

Figure 3. Box and whisker plots of the median $\delta^{15}N$ in *Sargassum mangarevense* tissue across three treatments from a short-term transplant experiment, showing the third quartile (Q3) and first quartile (Q1) range of the data, the whiskers (95% quartile) and data outliers. Connecting letters indicate significance between treatments. Stable isotopic signatures were measured in subset samples of the same specimens that were collected from a low-nutrient reef (Initial), placed in laboratory aquaria to deplete internal nutrient stores for ~7 days (Pre-Transplant), before they were deployed on the bleached reef for 3 weeks (Post-Transplant) (n=10).