

Reviewer Comment	Author Response	New location
Referee #1		
General comment		
<p>This study assessed the impact of mangrove dieback and recovery through assessing the changes in vegetation population and biogeochemical variables in the Gulf of Carpentaria. Findings from this study are important to understand the impact of mangrove disturbance on the biogeochemical processes, specifically their interaction between plant and sediment. This study will contribute to the current blue carbon literature while such coastal ecosystems are expected to undergo extreme disturbance in future. The manuscript is well structured and nicely written but can still be improved for some minor correction. Also, I would suggest providing further raw dataset obtained from this study in the supplementary information or via digital data repository platforms such as Mendeley Data and Figshare. Such of these data will provide a better understanding for the readers and also be useful for future meta-analysis based study on this topic. The publication of the ms can be recommended after revisions.</p>	<p>We thank the reviewer for the constructive feedback on the manuscript and have now modified it to clarify the points raised.</p> <p>As suggested, we have now provided additional dataset in the supplementary information for a better understanding for the readers. Table S1, S2, S3 and S5.</p>	
Minor comments		
<p>Line 15: I would suggest defining the acronym for C, N, S when they first appeared. Sometimes acronyms can make confusion for non-specialist readers.</p>	<p>We have now defined the acronym for C, N, S at the first appearance.</p>	Ln 16
<p>Line 19: Were these samples or applicable for vegetation and sediments only?</p>	<p>The samples include invertebrates, plants and sediments. We have now changed the sentence to clarify this (blue is new text).</p> <p>“Invertebrates and associated organic matter including mangroves, and sediments from the impacted ecosystem showed enrichment in ¹³C, ¹⁵N and ³⁴S relative to those from an adjacent unimpacted reference ecosystem...”</p>	Ln 19-21
<p>Line 25: It would be great if data on vegetation population increase are presented in the abstract.</p>	<p>We agree. We have now provided vegetation data in the abstract. “The seedling density increased from 0.2 per m² in 2016 to 7.1 per m² in 2018 in the impacted forest”.</p>	Ln 25-26
<p>Lines 51-55: Most of the cases provided here highlight the impact of mangrove loss. If possible, authors can provide example or reference how mangrove recovery may restore biogeochemical processes. It is important when one of the study aims is to</p>	<p>We have inserted new sentences and references to improve this section.</p> <p>“Although mangroves can recover from mortality events, the rate of recovery can be slow. For example, a study of mangrove</p>	Ln 45-48

document the ecosystem recovery profile following dieback.	mortality attributed to an oil spill incident shows full recovery may take over 50 years (Connolly et al. 2020) and full recovery of belowground C and N stocks after mangrove replantation may take over 40 years (Adame et al., 2018)".	
Line 100: 'Three field campaigns were carried out in August 2016, 2017 and 2018'. This sentence is redundant with lines 90-91.	We have now removed the sentence.	
Line 115: Does this mean that leaves from the impacted site were obtained from seedling rather than survived mature trees?	Leaves were from regrowth from survived trees. We have now changed the sentence to clarify this. "In the impacted site, leaves were collected from regrowth of trees that had survived"	Ln 124 - 125
Line 116: I would suggest describing further steps on wood sampling approach, whether samples were done for sapwood only or with heartwood as well?	Samples were from sapwood. We have now added more information on wood sampling. "wood samples (n=2, 5 to 25cm diameter) were collected using a hand saw from stems at chest height from the mid intertidal zone of each forest. Dead trees were sampled at the impacted site. Two to three bulk SIA measurements were made from sapwood (2 to 3cm deep) of each sample and measurements were averaged."	Ln 126-128
Line 117: It is quite hard to see which stable isotope is applied for each sample. It would be great if the raw data are provided in Supplementary Information or online database.	We have now provided additional data in the supplementary information (Table S1, S2, S3 and S5).	
Line 120: In this section, maybe the readers want to know the reason for having a surface (<0.5 cm) and subsurface (0.5-20 cm) sediment samplings.	The reason for having sediment samples from two depths is to compare surface sediments that represent the recent deposition and microphytobenthos, with the subsurface fraction which represents a long-term average. We have now reworded the sentence to clarify this. "In 2018, surface (<0.5 cm) sediments that represent the recent deposition and microphytobenthos (MPB) were collected along each transect. Additionally, subsurface (0.5 to 20 cm) sediment samples (n=6) that represent long-term averages were collected at the mid intertidal zone of each forest using a core sampler (5 cm in diameter and 20 cm deep)".	Ln 131-133
Line 121: 'each forest' do you mean each zone? How many soil core per zone?	Sediment cores were independent samples from the surface sediment. Samples (n=2 per transect) were collected from the mid intertidal zone. We have now changed the sentence to clarify this. "Additionally, subsurface (0.5 to 20 cm) sediment samples	Ln 132-133

	(n=6) that represent long-term averages were collected at the mid intertidal zone of each forest using a core sampler (5 cm in diameter and 20 cm deep)".	
Line 133: Was number of the sample here denotes the number of photographs or number of quadrats? How many quadrats per forest zone at each transect?	A photo was taken for each quadrat, so the number of photos and number of quadrats are the same. We have now inserted " To estimate mangrove seedling/sapling densities (ind. m ⁻²) from each forest and their changes over time, seedling/saplings were counted with a 50 x 50 cm quadrat at the mid intertidal zone. A photo was taken of each quadrat (for 2016, n=124 for the unimpacted forest and n=143 for the impacted forest, for 2017, n=161 and n=175, and for 2018, n=80 and n=117, respectively) and then counts of seedlings and samplings were made in the laboratory".	Ln 143-146
Line 191: Was the variation similar to the impacted site? re: 34S depleted from higher to the lower tidal zone	Yes, in both forests, leaf $\delta^{34}\text{S}$ values decreased from the higher to lower intertidal zones. This is shown in the Fig 3 and the data is provided in the supplementary information.	Fig 3
Line 259: Double increased? Here may worth to discuss why both unimpacted and impacted sites show similar mangrove seedling increase, despite they have with different number and rates.	We have now discussed this in more detail. " In both mangrove forests at the Gulf of Carpentaria site, the density of mangrove seedling/samplings significantly increased throughout the period from 2016 to 2018, suggesting that recovery was starting to occur in some areas within 32 months after the dieback and propagule pool was available in the vicinity. The increase in seedling/sampling density at the unimpacted site was unexpected, but this indicates that there was some stress at the unimpacted site during the dieback period and/or the temporal variability of seedling/samplings density was high at the site."	Ln 264-269
Line 271: In related to Kelleway et al 2018, was 13C between leaf and wood different significantly from this dieback study?	It seems like the wood samples are more enriched than the leaves, but we do not have enough wood samples to make this comparison and also the wood samples were independently sampled from the leaves.	
Line 324: 'lower mangrove C inputs' change mangrove with autochthonous?	We have now changed "mangrove" to "autochthonous".	Ln 329
8Line 326: 'The surface sediment (0 - 0.5 cm) differed relatively more than the deeper (0.5 to 20 cm) fraction' Sorry, it is quite hard to follow this sentence.	We have now changed the sentence. "The surface sediment varied more than the subsurface fraction".	Ln 331
Line 328: How about C/N ratio? It would be great to explore further roles of C/N ratio	Thank you. We have now provided the C/N ratio data in Table 2. The result was discussed in the text. "Despite the substantial variation	Ln 215-216

to support the findings in addition to elemental and isotope variation.	in TOC and TN, the C/N ratio did not differ significantly between the two sites (ANOVA $P > 0.05$)”.	
Table 1: Thanks. This table is really helpful to understand the scattered sampling time and what was sampled. Table 2: it is quite unusual to have a comma between mean and SD. I would suggest replacing the comma with \pm here and elsewhere.	We have now used \pm instead of comma between mean and SD.	Table 2 Table 3 Table 4
Figure 2: In the graph, I would suggest providing seedling per hectare instead of per quadrat.	Thank you, we agree. Since the size of the quadrat is very small compared with a hectare, we have now used seedling per m ² in the figure.	Figure 2
Figure 3: Were the authors collect the wood sample as well for SIA? Is there a possibility of presenting ¹³ C and ¹⁵ N in the same way with ³⁴ S, from landward to seaward?	Wood samples were only collected from the mid intertidal zone, so we cannot present the data in the same way.	
Figure 7: It is a nice conceptual figure. Please clarify if isotopes denote for both plant and sediment.	We have now indicated this in the figure “ δ represents the isotope values of animals, plants and sediment ”.	Figure 7
Referee #2 Martin Zimmer		
General comment		
The authors provide data from element and stable isotope analyses in order to better understand post-die-off dynamics of a mangrove ecosystems. They interpret an observed enrichment in heavier isotopes as indicators of reduced C and N fixation and reduced S reduction in the impacted mangrove stand, while the increasing number of mangrove recruits over time suggests recovery of the vegetation. The lack of recovery of CNS cycling after 32 months, by contrast, is considered an indicator for the biogeochemical legacy of the mass mortality event.	We thank Dr Martin Zimmer for the constructive feedback on the manuscript.	
Introduction: The praise of the stable isotope approach should certainly also include some mentioning of its flaws and weaknesses. Among these, the changes in the isotopic signature are not as globally "predictable" as the first paragraph of the Introduction suggests: many of these changes do not only depend on the species (both consumer and resource) involved but also on the specific environmental conditions: : : I suggest the first and second paragraph be merged (as they state essentially the same), following a first paragraph of extreme events (currently 2nd paragraph).	We agree. We have now mentioned the flaws and weaknesses of the stable isotope approach in the introduction and in the interpretation of these results in the discussion. We have now merged also the first paragraph and the third paragraph to provide one paragraph of the stable isotope approach, following the paragraph of extreme events. “Stable isotope analysis (SIA) provides biogeochemical process information integrated over time and is useful for environmental assessment and monitoring. As elements such as carbon (C), nitrogen (N) and	Ln 50-67

	<p>sulfur (S) circulate in the biosphere, stable isotopic compositions of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$ can change in predictable ways due to mixing and fractionation, giving insights into sources and cycling of these elements (Fry 2006). SIA has been widely used in mangrove ecosystem studies to better understand food web interactions (Bouillon et al., 2008; Larsen et al., 2012; Bui and Lee, 2014; Abrantes et al., 2015), nutrient uptake (McKee et al., 2002), water use (Santini et al., 2015; Hayes et al., 2019), cycling of C (Maher et al., 2013a; Maher et al., 2017; Sasmito et al., 2020), N (Fry and Cormier, 2011), S (Raven et al. 2019), and greenhouse gas emissions (Maher et al., 2013b). While traditional field methods such as measuring species composition to evaluate structure and functioning of ecosystems can be time-consuming and expensive, SIA of ecosystem components can evaluate functional aspects of element cycling and food webs in a cost-effective way (Fry, 2006). To quantify food web dynamics, SIA of total organic matter (“bulk”) requires determination of the baseline isotope values of the food web, but this is difficult to achieve, particularly for complex detrital food webs. Some of the uncertainties associated with bulk SIA have been clarified by compound-specific isotope analysis of amino acids (CSIA-AA). This technique has been increasingly used to assess pathways of energy transfer throughout food webs by distinguishing the effects of baseline isotope values and trophic transfer. Distinct isotopic fractionation between two groups of amino acids occurs with each trophic transfer. In general, non-essential and/or trophic AAs show large isotopic fractionation per trophic step, while essential and/or source AAs show little fractionation, reflecting the baseline isotope values of the food web (Ishikawa et al., 2018; Larsen et al., 2013; Ohkouchi et al., 2017).”</p>	
<p>Methods: Before learning about the die-back event (and hypotheses on its causes), I would like to get some information about the mangroves themselves, such as species composition, forest structure and so on! It seems <i>Avicennia marina</i> is/was the predominant species in the study area.</p>	<p>We have now provided some more information on the characteristics of the mangrove forest studied before providing the information of the die-back event.</p> <p>“The Gulf of Carpentaria in tropical Northern Australia is an extensive, shallow coastal gulf. The area mainly consists of low-lying</p>	<p>Ln 79-87</p>

	<p>wetlands and is largely inaccessible with little direct human activity. Mangroves are abundant in the area, but the dry climate limits the extent, diversity, and height of mangroves in the region (Asbridge et al., 2016). The wide tidal wetlands spread along the shoreline with high intertidal saltpans and saltmarsh covering more area than mangroves. Mangroves are often distributed in the seaward margin, typically as a narrow strip and fronted by extensive, shallow mudflats. The distribution of mangroves in this region is associated with tidal and freshwater inundation, river discharge and regular sediment supply through freshwater input. Increased amounts of rainfall and associated flooding and sea level rise were responsible for recent mangrove extension in this region between 1987 and 2014 (Asbridge et al., 2016). <i>Avicennia marina</i> was the dominant mangrove species at the study site in Karumba, Gulf of Carpentaria (Fig 1A)”.</p>	
<p>It is interesting that hypersalinization (as a result of drought) is mentioned as major causative agent of the mass mortality. As <i>A. marina</i> is known to also occur under quite adverse conditions (e.g., at distribution limits of mangroves), wouldn't we assume that it is as tolerant to salinity stress as, e.g., <i>A. germinans</i> from the AEP? It would be nice to get at least an idea of the sediment salinity this hypersalinization resulted in. The reader might also be highly interested in understanding why the mangrove stand north of the river mouth was impacted, while the nearby(!) stand south-west of the river mouth was not.</p>	<p>We have now discussed the cause of dieback in more detail.</p> <p>“The dieback coincided with a weak monsoon (low rainfall), combined with high vapor pressure deficit, and El Niño–Southern Oscillation-induced low sea-levels (Duke et al., 2017; Lovelock et al., 2017; Harris et al., 2017). The drought conditions most likely caused accumulative hydric, thermal and radiant stresses (Duke et al., 2017). In addition to low water availability, iron (Fe) toxicity due to a rapid mobilisation of sedimentary Fe and regional variability in groundwater flows may have also played a role in the dieback (Sippo et al., 2020a)”.</p> <p>We can only hypothesize as to why the mangrove stand north of the river mouth was impacted while stand south-west of the river is not. We have now inserted “ Some local factors (e.g. river influence and localized groundwater flow paths) may have kept some mangroves from dying back in the region”</p>	<p>Ln 95-99</p> <p>Ln 104-105</p>
<p>It is obvious that 3 transects were monitored in each of the two stands – how many sampling plots were established in each transect? How were the data from these plots handled (pooled?, : : :?)? We need to better</p>	<p>The number of sampling plots varied among samples. For example, 5 plots for mangrove leaves and 6 plots for sediment along the tidal zone. Data from these plots were pooled. To clarify the spatial details of the sampling</p>	<p>Table 1</p>

understand the (spatial) details of the sampling design!	design, we have now added a table with number of plots for each analysis.	
Some more details about the "wood samples" would be helpful: how deep? where on the stem? Etc: : :	We have now added more detail about the wood samples. "wood samples (n=2, 5 to 25cm diameter) were collected using a hand saw from stems at chest height from the mid intertidal zone of each forest. Dead trees were sampled at the impacted site. Two to three bulk SIA measurements were made from sapwood (2 to 3cm deep) of each sample and measurements were averaged."	Ln 126-128
According to the hydrodynamics of the area, do the offshore water samples reflect material that is likely to be washed into the mangroves or to be derived from the mangroves?	The mangrove area is adjacent to an extensive area of mudflats. Material derived from the mangrove area is likely diluted and the offshore water samples mostly reflect material that is likely to be washed into the mangrove such as POM and phytoplankton. We have now inserted "...particulate organic matter (POM), material such as phytoplankton that is transported to the mangrove".	Ln 140-141
How were the photos taken to allow for relating the number of the seedlings on the photo to a given (unit of) area?	For each photo, a 50cm x 50cm of quadrat was used to indicate a unit of area. "To estimate mangrove seedling/sapling densities (ind. m ⁻²) from each forest and their changes over time, seedling/saplings were counted with a 50 x 50 cm quadrat at the mid intertidal zone. A photo was taken of each quadrat (for 2016, n=124 for the unimpacted forest and n=143 for the impacted forest, for 2017, n=161 and n=175, and for 2018, n=80 and n=117, respectively) and then counts of seedlings and samplings were made in the laboratory."	Ln 143-146
Even though the transects were chosen as to render the sites for comparison as similar as possible, there remains the fact that "unimpacted" and "impacted" are not replicated – strictly speaking, we are comparison two sites, one of which is by chance impacted, the other one is not. In this very particular case, I don't consider this a real issue, as the difference is very clear, but I would like to see that the authors take this non-replicated comparison of two sites that than results in generalized conclusions on "impacted" versus "unimpacted" into account	We agree. We have now mentioned this in the conclusion. "Although the unimpacted and impacted forests were not replicated in this study, the difference between the two sites was clear"	Ln 402-403

and at least mention this restriction to their conclusions.		
Results: "had a 34S value of 16.6‰. . . compared to which value for the unimpacted site?"	Wood samples for the unimpacted site did not have enough S to determine the isotope values, therefore we do not have sufficient data to make this comparison and have now removed the wood 34S values to avoid confusion.	
l:225 - 230 : these values do not seem to be SIGNIFICNATLY different; though?	Figure 5 shows the ANOVA results and which samples significantly differed.	
l:230 ff(and throughout) : what is the "forest type" here? I think we are just comparing one impacted and one unimpacted stand (not two forest types); and I suggest to stick to this (like above)!	We have now used impacted and unimpacted throughout the ms.	
l:236 as above (and throughout) - - is "consistently" significant? It doesn't look as if it is(except for 2018). . . If the values are not significantly different ; we cannot consider them" different"; - please clarify!	We have now modified the line and indicated which means are significantly different in the text. "In 2018, leaf feeder $\delta^{13}\text{C}$ values, grazer $\delta^{34}\text{S}$ values and algae feeder $\delta^{34}\text{S}$ values significantly differed between the impacted and unimpacted sites (Turkey post hoc test, $P < 0.05$), showing no recovery of the invertebrate fauna $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ status 32 months after the dieback event. However, $\delta^{15}\text{N}$ values became similar between the two forests in 2018 (Fig. 5)".	Ln 243-246
Very minor linguistics: l.181: "than at the unimpacted site" l.183: "dominant mangroves species, <i>A. marina</i> , did not differ" l.211: "than those from the unimpacted site" l.218: "was similar to value of those collected in the mudflat"	Thank you. We have now corrected the linguistic errors.	
Discussion: "mangrove degradation may be followed by fast colonisation of nonmangrove herbaceous species" – this is an important statement on a general and global problem: in the Caribbean, <i>Acrostichum aureum</i> , the Golden mangrove fern, builds up a dense canopy in disturbed/clear-felled mangrove areas. As this species, as well as congenics, also occur in the IWP: was the impacted forest (re-)colonized by the fern, or is there no propagule pool available in the vicinity?	The impacted site was not colonized by the fern. There was fast colonisation by mangroves, so it is likely that a propagule pool was available in the vicinity. We have now inserted "Further, mangrove degradation may be followed by fast colonisation of non-mangrove herbaceous species, e.g. succulent saltmarsh (<i>Mbense</i> et al., 2016; McKee et al., 2007; Rashid et al., 2009). However, this was not largely evident from our study site. In both mangrove forests at the Gulf of Carpentaria site, the density of mangrove seedling/samplings significantly increased throughout the period from 2016 to 2018, suggesting that recovery was starting to occur in some areas within 32 months after	Ln 262-267

	the dieback and propagule pool was available in the vicinity”.	
1.265: why would the "stomatal conductance" be reduced in the impacted site? The environmental conditions were very similar (c.f. Methods), while one site showed mass mortality and the other one did not – what actually is/was the (environmental) difference between these two sites? Why did the mangroves die here but not there? Is the biogeochemical pattern observed a legacy of the die-back, or might it be related to the reason for the die-back (while a nearby mangrove did not exhibit mass mortality)? Several potential reasons for the observed ^{13}C pattern are listed – don't the authors want to discuss these?	We have now discussed several potential reasons for the observed ^{13}C pattern in more detail. “Overall, ^{13}C -enriched leaf $\delta^{13}\text{C}$ values in the impacted forest likely suggest that there are chronic stresses associated with the dieback event that reduced stomatal conductance. Such environmental stresses may include hypersalinization of sediments and hydric, thermal and radiant stresses following canopy losses that cause higher evaporation and lower water availability. The leaves at the unimpacted site were largely depleted in ^{13}C , suggesting that there was higher water availability at the unimpacted site, possibly associated with regional variability in groundwater flows, e.g., Sippo et al. (2020)”.	Ln 284-289
1.275: what might these "chronic stresses" be? Are they a consequence of the die-back, or are they the reason (the drought that seems to have caused the mass mortality can probably not be considered a "chronic stress" but rather a massive disturbance)?	We have now inserted “Such environmental stresses may include hypersalinization of sediments and hydric, thermal and radiant stresses following canopy losses that cause higher evaporation and lower water availability”.	Ln 285-289
1.289: this is very interesting! I would have expected lower rather than higher variability in (sediment/microbial) processes upon such string disturbance – can you expand on this to explain how/why the drought and/or die-back would increase the variability of processes?	We have now inserted “The higher variability in leaf $\delta^{15}\text{N}$ in the impacted forest suggests higher variability in processes affecting the $\delta^{15}\text{N}$ status of available N. For example, changes to sediment conditions including redox transitions and soil moisture content following the dieback may have affected microbial processes of N, whereas the unimpacted forest may have had more stable sediment conditions and N processes”.	Ln 299-302
1.315: this interpretation of the findings suggests that at the impacted site it was dead wood that was sampled (from standing dead stems?), whereas wood from living trees was sampled at the unimpacted site – is that correct?	Yes, we sampled dead wood from the impacted site and living wood from the unimpacted site. We have now mentioned this in the ms. Since we do not have sufficient S isotope data from the two forests for comparisons, we have deleted S isotope data and also the interpretation to simplify the ms and avoid confusions.	
Fauna: before we can go into this discussion, the above issue of whether "consistent"/"substantial" is "significant" needs be clarified. Only IF the values are significantly different, it will make sense to discuss or interpret such differences!	We have now used “significant” to indicate which samples statistically differed.	
1.356: I don't follow this line of argument: Bui & Lee (2014) stress a potential enrichment by up to 5 – here we have a	We have modified the lines to clarify this.	

<p>difference of 6-7 ‰ : : is this sufficient to indicate "some additional contributions"?</p>	<p>“Typical mangrove leaf-eating sesarimid crab species generally have tissue $\delta^{13}\text{C}$ values within about +5‰ from mangrove detritus (Bui and Lee, 2014)”.</p> <p>“The leaf feeders were relatively depleted in ^{13}C, with $\delta^{13}\text{C}$ values of about -21 to -18‰, likely due to some use of mangrove leaves”.</p>	<p>Ln 352-353</p> <p>Ln 358-359</p>
<p>1.363: does that mean that mangrove leaves did not play a role as food source in BOTH forests? If so, this cannot be an effect of the mass mortality, and –of course we would then not expect any change over time, as this observation would have nothing to do with mangrove recovery after disturbance: : :</p>	<p>We consider that mangrove leaves played a minor role as food source, but MPB played a more important role in both forests. However, the presence or absence of mangroves can still change the isotope values of consumers, consistent with the finding for other studies, e.g., Bernardino et al. (2018).</p> <p>We have mentioned this in the text. “These findings did not support changes to feeding dependency following mangrove loss but suggested that the overall differences in the consumer bulk $\delta^{13}\text{C}$ values were most likely driven by differences in the resource organic matter $\delta^{13}\text{C}$ values e.g. changes to MPB $\delta^{13}\text{C}$ values that were likely associated with lower mangrove C fixation/respiratory inputs following the mangrove mortality. Furthermore, such findings indicate that the reported substantial change to the mangrove benthic faunal assemblage following the mangrove loss (Harada et al., 2019) was probably driven more by modification of physical habitat structure than changes in the use of food resources”.</p>	<p>Ln 392-397</p>
<p>1.395: I don’t understand "can reflect consumer tissues with little isotope effect" – how do the patterns in producers reflect patterns in consumers; shouldn’t it be the other way round?</p>	<p>We have modified the line. “The $\delta^{13}\text{C}_{\text{CAA}}$ patterns in producers, especially those of essential amino acids ($\delta^{13}\text{C}_{\text{EAA}}$), can be reflected in consumer tissues with little isotope effect”.</p>	<p>Ln 386-387</p>
<p>1.403: what is it that mostly affect MPB? Besides the biotic changes, we would expect much more light, and thus, higher evaporation and less water at the impacted than at the unimpacted site. This already will change MPB drastically.</p>	<p>Source of carbon and isotope fractionation can affect the isotope value of MPB.</p> <p>We have inserted “In our case, MPB $\delta^{13}\text{C}$ values changed significantly, likely due to changes to organic matter respiratory inputs and/or altered light environment and soil moisture contents that may change isotopic fractionation during carbon fixation”.</p>	<p>Ln 346-348</p>
<p>1.425: I do not understand how you derive these scenarios from the present study? I kind of agree with these potential scenarios (there might be other possibilities), but how does this relate to, how is this justified by, the present study?</p>	<p>These are likely scenarios and there might be other possibilities. What we have learned from this study is that biochemical changes can be reflected in the isotopic values of organisms. Multi-annual sampling can be used to track their changes overtime and such</p>	<p>Ln 424-425</p>

	<p>isotopic information can be used to monitor biogeochemical changes in the future. It can be expected from this study that when the impacted forest is fully recovered, it would be isotopically similar to the unimpacted site. If the forest is unable to recover this may not be observed.</p> <p>We have now inserted “While these are likely scenarios and there might be other possibilities, comparing the impacted forest and an adjacent unimpacted forest can help us quantify the recovery”.</p>	
<p>Minor: 1.410: omit "-"</p>	<p>We have now omitted “-“</p>	

Stable isotopes track the ecological and biogeochemical legacy of mass mangrove forest dieback in the Gulf of Carpentaria, Australia

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15 **Abstract.** A combination of elemental analysis, ~~bulk~~ ~~and~~ stable isotope analysis (~~bulk~~ SIA) ~~and compound-specific stable isotope analysis of amino acids (CSIA-AA)~~ was used to assess and monitor ~~carbon (C)C~~, ~~nitrogen (N)N~~ and ~~sulfur (S)S~~ cycling of a mangrove ecosystem that suffered mass dieback of trees in the Gulf of Carpentaria, Australia in 2015-16, attributed to an extreme drought event. Three field campaigns were conducted over a period from 2016 to 2018, at 8, 20 and 32 months after the event ~~to obtain biological time-series data.~~ ~~Samples including invertebrates, mangroves, and sediment were analysed for~~

20 ~~CNS elemental and isotopic compositions including compound-specific stable isotope analysis (CSIA) of amino acid carbon.~~ ~~Samples Invertebrates and associated organic matter including mangroves, and sediments from collected from~~ the impacted ~~ecosystem~~ecosystem showed enrichment ~~m~~ ~~were enriched~~ in ¹³C, ¹⁵N and ³⁴S relative to those from an adjacent unimpacted reference ecosystem, likely indicating lower mangrove carbon fixation, lower nitrogen fixation and lower sulfate reduction in the impacted ecosystem. For example, invertebrates representing the feeding types of grazing, leaf feeding, and algae feeding

25 were more ¹³C enriched at the impacted site, by 1.7 – 4.1‰ and these differences did not change over the period from 2016 to 2018. The CSIA-AA data indicated widespread ¹³C enrichment across five essential amino acids and all groups sampled (except filter feeders) within the impacted site. ~~The Mangrove seedling density and sapling populations increased substantially~~ from ~~in~~ ~~0.2 per m² in~~ 2016 to ~~7.1 per m² in~~ 2018 in the impacted forest, suggesting recovery of the ~~mangrove~~vegetation. Recovery of CNS cycling, however, was not evident even after 32 months, suggesting a biogeochemical legacy of the mortality

30 event. Continued monitoring of the post-dieback forest ~~is required to would help to~~ predict the long-term trajectory of ecosystem recovery. ~~This study shows that time-series In such long-term monitoring programs, SIA can that can~~ track biogeochemical changes over time ~~and can help to evaluate detect underlying biological mechanisms that drive changes and~~ recovery of ~~anthe~~ impacted mangrove ecosystem ~~from an extreme event.~~ ~~To gain further insight, our use of CSIA can help~~ ~~show feeding dependencies in mangrove food webs and their response to disturbances.~~

1 Introduction

~~Stable isotope analysis (SIA) is a powerful tool for environmental assessment and monitoring that provides information about biogeochemical source and processes over time. As elements such as carbon (C), nitrogen (N) and sulfur (S) circulate in the biosphere, stable isotopic compositions of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$ change in predictable ways due to mixing and fractionation, giving insights into sources and cycling of these elements (Fry 2006). SIA has been widely used in mangrove ecosystem studies to better understand food web interactions (Bouillon et al., 2008; Larsen et al., 2012; Bui and Lee, 2014; Abrantes et al., 2015), mangrove nutrient uptake (McKee et al., 2002), mangrove water use (Santini et al., 2015; Hayes et al., 2019), cycling of C (Maher et al., 2013a; Maher et al., 2017; Sasmito et al., 2020), N (Fry and Cormier, 2011), S (Raven et al. 2019), and greenhouse gas emissions (Maher et al., 2013b).~~

Low frequency, high intensity weather events, such as droughts, tropical cyclones, heatwaves and climatic extremes can cause mass mortality of foundation species such as mangroves (Sippo et al., 2018), saltmarshes (Silliman et al., 2005), seagrasses (Thomson et al., 2015), kelps (Wernberg et al., 2016) and corals (Hughes et al., 2017). The frequency and intensity of extreme climatic events are expected to increase due to climate change (Coumou and Rahmstorf, 2012; Stott, 2016). In 2015-16, an extensive area (>7000 ha) of mangrove forest along ~1,000 km of coastline in the Gulf of Carpentaria, Australia, experienced severe dieback, an event associated with the climatic extreme of drought (Duke et al., 2017; [Sippo et al., 2020a](#)). As mangroves show characteristics of pioneer species (Tomlinson, 2016), large-scale disturbances have likely played an important role in their evolution. However, the processes, rates and patterns of recovery from disturbances are still largely unknown. In most cases, recovery of mangroves primarily relies on the recruitment of seedlings (Smith et al., 1994; Krauss and Osland, 2019). Disturbances in mangrove forests not only affect recruitment, but can also change the cycling of C, N and S. Loss of mangrove trees and root structures can change organic matter inputs, sediment oxygenation and degradation of sediment organic matter. ~~These changes alter overall sediment conditions,~~ with consequences for benthic assemblages (Sweetman et al., 2010; Bernardino et al., 2018; Harada et al., 2019), [coastal carbon cycle \(Jeffrey et al., 2019; Sippo et al., 2020b\)](#), sediment C and N stocks (Adame et al., 2018), microbial assemblages, and associated nutrient processes e.g. nitrogen fixation and sulfate reduction (Sjöling et al., 2005). [Although mangroves can recover from mortality events, the rate of recovery can be slow. For example, a study of mangrove mortality attributed to an oil spill incident shows that full canopy recovery may take over 50 years \(Connolly et al. 2020\). Full recovery of belowground C and N stocks may take over 40 years after mangrove replantation \(Adame et al., 2018\).](#)

~~Stable isotope analysis (SIA) provides biogeochemical process information integrated over time and is useful a powerful tool for environmental assessment and monitoring that provides information about biogeochemical source and processes over time.~~

As elements such as carbon (C), nitrogen (N) and sulfur (S) circulate in the biosphere, stable isotopic compositions of $^{13}\text{C}/^{12}\text{C}$, $^{15}\text{N}/^{14}\text{N}$ and $^{34}\text{S}/^{32}\text{S}$ can change in predictable ways due to mixing and fractionation, giving insights into sources and cycling of these elements (Fry 2006). SIA has been widely used in mangrove ecosystem studies to better understand food web interactions (Bouillon et al., 2008; Larsen et al., 2012; Bui and Lee, 2014; Abrantes et al., 2015), mangrove nutrient uptake (McKee et al., 2002), mangrove water use (Santini et al., 2015; Hayes et al., 2019), cycling of C (Maher et al., 2013a; Maher et al., 2017; Sasmito et al., 2020), $-\text{N}$ (Fry and Cormier, 2011), S (Raven et al. 2019), and greenhouse gas emissions (Maher et al., 2013b). While

~~Traditional field methods such as measuring species composition to evaluate structure and functioning of ecosystems include measures of species composition, but these field assessments can be time-consuming and expensive, and may not provide enough quantitative information about system functioning (e.g. Kling et al., 1992). SIA of ecosystem components can be a powerful way of quantitatively evaluating functional aspects of element cycling and the health of ecosystems (e.g. integrity of the food webs in a cost-effective way (Fry, 2006). To quantify food web dynamics, SIA of total organic matter (“bulk”) requires determination of the baseline isotope values of the food web, but this is difficult to achieve, particularly for complex detrital food webs. Some of the uncertainties associated with bulk SIA have been clarified by compound-specific isotope analysis of amino acids (CSIA-AA). This technique has been increasingly used to assess pathways of energy transfer throughout food webs by distinguishing the effects of baseline isotope values and trophic transfer. Distinct isotopic fractionation between two groups of amino acids occurs with each trophic transfer. In general, non-essential and/or trophic AAs show large isotopic fractionation per trophic step, while essential and/or source AAs show little fractionation, reflecting the baseline isotope values of the food web (Ishikawa et al., 2018; Larsen et al., 2013; Ohkouchi et al., 2017). have become widespread due to the relative ease and low cost of sample preparation and analysis (Fry, 2006). Compound specific stable isotope analysis (CSIA) is increasingly employed as a complementary tool to bulk SIA. For instance, while bulk SIA of C, N and S provide an overview of food webs (Bouillon et al., 2008), CSIA of amino acids (AAs) help measure details of organic matter cycling (Ishikawa et al., 2018; Larsen et al., 2013; Ohkouchi et al., 2017)~~

We investigated changes in C, N and S cycling associated with the Gulf of Carpentaria mangrove forest dieback (Duke et al., 2017), using a combination of traditional ecological survey techniques, bulk SIA, and CSIA-AA of amino acid-carbon. We hypothesised that the mortality of mangrove foundation species has changed the overall circulation of C, N and S elements and these biogeochemical changes would most likely be reflected in $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of mangrove ecosystem components such as mangrove plants, sediment and associated animals. We also tested the hypothesis that these isotopic compositions changed over time with the recovery of mangrove vegetation. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values were measured for samples including mangroves, sediment and invertebrates collected in a comparative setting of impacted mangrove forest site and an adjoining unaffected reference forest site in the Gulf of Carpentaria, Australia.

2 Material and Methods

100 2.1 Study site

~~The Gulf of Carpentaria in tropical Northern Australia is an extensive, shallow coastal gulf. The area mainly consists of low-lying wetlands and is largely inaccessible with little direct human activity. Mangroves are abundant in the area, but the dry climate limits the extent, diversity, and height of mangroves in the region (Asbridge et al., 2016). The wide tidal wetlands spread along the shoreline with high intertidal saltpans and saltmarsh covering more area than mangroves. Mangroves are often distributed in the seaward margin, typically as a narrow strip and fronted by extensive, shallow mudflats. The distribution of mangroves in this region is associated with tidal and freshwater inundation, river discharge and regular sediment supply through freshwater input. Increased amounts of rainfall and associated flooding and sea level rise were responsible for recent mangrove extension in this region between 1987 and 2014 (Asbridge et al., 2016). *Avicennia marina* was the dominant mangrove species at the study site in Karumba, Gulf of Carpentaria (Fig 1A).~~

110 Over 7,000 ha of mangroves along ~ 1,000 km of the Gulf of Carpentaria coastline in Australia experienced mass mortality during the summer in 2015-16, (Duke et al., 2017), the most extensive mangrove forest dieback ever recorded due to natural causes (Sippo et al., 2018). At the same time, there were coinciding mangrove mass mortality events in Exmouth, Western Australia (Lovelock et al., 2017) and Kakadu National Park, Northern territory (Asbridge et al., 2019). The climate in the Gulf region is wet-dry tropical with mean annual precipitation ranging from approximately 600 to 900 mm. Dry conditions prevail for six to eight months and most rainfall occurs between December and March (Bureau of Meteorology, see www.bom.gov.au). ~~The climatic conditions limit the extent of mangroves in the region (Asbridge et al., 2016).~~The dieback ~~was coincided with most likely linked to~~ a weak monsoon (low rainfall), combined with high vapor pressure deficit, and El Niño–Southern Oscillation-induced low sea-levels (Duke et al., 2017; Lovelock et al., 2017; Harris et al., 2017; ~~Harris et al., 2018~~). The ~~drought conditions se conditions~~ most likely ~~resulted in hypersalinization and~~ caused accumulative hydric, thermal and radiant stresses (Duke et al., 2017). ~~In addition to low water availability, iron (Fe) toxicity due to a rapid mobilisation of sedimentary Fe and regional variability in groundwater flows may have also played a role in the dieback (Sippo et al., 2020a; Lovelock et al., 2017; Harris et al., 2017; Harris et al., 2018).~~ The event led to the widespread death of mangrove trees in the region providing ~~an unfortunate yet a~~ unique opportunity to test tree mortality effects on biogeochemical and ecological functioning of mangroves and capture recovery patterns.

125 Three field campaigns were conducted in August 2016 (8 months after the event), August 2017 (20 months after the event) and August 2018 (32 months after the event) in the winter dry-seasons in Karumba, ~~Gulf of Carpentaria, Australia (Fig. 1A)~~. Some local factors (e.g. river influence ~~and localised groundwater flow paths~~) may have kept some mangroves from dying back in the region. A forest that had suffered dieback (impacted) on the east of Norman ~~river~~ River outlet and an adjoining unaffected forest (unimpacted) on the west, provided the setting for comparisons. ~~*Avicennia marina* was the dominant~~

~~mangrove species.~~ In order to assess differences between the two forests (impacted vs. unimpacted), as well as to capture trends from across the intertidal zone and to ensure that the physical-oceanographic conditions between the two forests were as similar as possible, three sampling transects (2 to 2.5 km apart) were set for each forest with the length of each transect being approximately 200 m (Fig. 1B, C). Each transect consists of six sampling plots (approx. 40m apart), namely forest edge (landward), high, mid, low, forest edge (seaward) and mudflat (Table 1). Samples from each plot were pooled for analysis. Due to logistical constraints and the presence of saltwater crocodiles, fieldwork was restricted to daytime, low tide and dry seasons.

2.2 Samples

~~Three field campaigns were carried out in August 2016, 2017 and 2018.~~ Since our focus was to measure recovery of mangrove vegetation and food-web, we monitored mangrove sapling/seedlings and stable isotopes of invertebrates during the period from 2016 to 2018. Mangrove and sediment samples were also collected but they are limited to 2018 (Table 1). Some of the SIA samples including invertebrates, mangrove and sediment collected in 2017 were used to measure the initial dieback reported in Harada et al. (2019).

During each field campaign, four common mangrove macroinvertebrate groups with different feeding modes were collected from each forest including a leaf-eating crab (*Parasesarma moluccensis* and/or *Episesarma* sp.), an algae-eating (deposit-feeding) crab (*Tubuca signata*), a grazer gastropod (*Telescopium telescopium*) and a filter-feeding bivalve (*Saccostrea* sp, an oyster). For each feeding group, 3 to 5 individuals at each of the sampling transects (n=3) within the forest were collected and muscle tissues were pooled for SIA.

In 2018, ~~we further divided each transect into five zones (50m apart), namely forest edge (landward), high, mid, low and forest edge (seaward).~~ Fully developed green leaves of *A. marina* were collected from at about 1 to 1.5 m height, from 3 to 5 individual trees (1 to 3 leaves per tree) at each sampling plot zone, stored in plastic containers, then composited. In the impacted site, ~~regrowth was occurring in some trees, and~~ leaves were collected from this regrowth of survived trees that had survived. Leaf samples were washed thoroughly, rinsed with distilled water and the main vein was removed. Additionally, wood samples (n=2, 5cm to 25cm diameter) were collected using a hand saw from stems at chest height from the mid intertidal zone of each forest. Dead trees were sampled at the impacted site. Two to three bulk SIA measurements were made from sapwood (2 to 3cm deep) of or each wood sample and measurements were averaged. Wood samples were generally very low in S and not sufficient only one wood sample had sufficient S for $\delta^{34}\text{S}$ analysis.

In 2018, surface (<0.5 cm) sediments that represent the recent deposition and microphytobenthos (MPB) were ~~as~~ collected along ~~the intertidal zones of~~ each transect ~~from the forest edge (landward) to forest edge (seaward) and also in the adjacent mudflat.~~ Additionally, in each forest, subsurface (0.5 to 20 cm) sediment samples (n=6) that represents a long-term averages

165 were collected at the mid intertidal zone of each forest using a core sampler, (5 cm in diameter and 20 cm deep). For $\delta^{13}\text{C}$ measurements, the sediment samples were acidified with 1M HCl to remove the inorganic fraction. Microphytobenthos (MPB) samples (n=6) were separated from surface sediment collected at each forest. The separation was achieved through density gradient centrifugation in Ludox colloidal silica (Sigma) as described in Bui and Lee (2014). The MPB extraction was followed by microscopic examination to confirm that samples mostly contained green cells (i.e. diatoms and filamentous cyanobacteria).
170 Additionally, surface sediment samples were collected from offshore (approx. 1km) using a grab sampler and from the adjacent saltpan (approx. 200m inland from the forest). Offshore water samples (n=3) were also collected and then filtered through glass fiber filters (diameter 44mm, pore size 0.7 μm , Whatman GFF) to obtain particulate organic matter (POM). material such as phytoplankton that is transported to the mangrove.

175 To estimate mangrove seedling/sapling densities (ind. m^{-2}) from each forest and their changes over ~~time~~the two-year period (2016 to 2018), seedling/saplings were counted with ~~a~~ 50 x 50 cm quadrats ~~at the mid intertidal zone. A~~ ~~In this process,~~ photographs ~~was~~ ~~ere~~ taken ~~for~~of each quadrat to give a unit of area in the field (for 2016, n=124 for the unimpacted forest and n=143 for the impacted forest, for 2017, n=161 and n=175, and for 2018, n=80 and n=17, respectively) and then counts of seedlings and samplings were made in the laboratory. The seedlings/samplings were mostly *A. marina* but also include some
180 *Aegiceras corniculatum*.

2.3 Stable isotope analysis

All invertebrate, leaf, sediment and filter samples were stored separately in sealed plastic containers at -20°C until analysis, then dried at 60°C , powdered, homogenized and put in tin capsules for SIA. $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ measurements were carried out on an elemental analyzer (Europa EA-GSL, Sercon) coupled to an isotope ratio mass spectrometer (Hydra 20-22, Sercon)
185 at Griffith University, Brisbane, Australia. Isotope values are reported relative to Vienna Pee Dee Belemnite (PDB), atmospheric N_2 (AIR), and Vienna Canyon Diablo Troilite (VCDT) for C, N and S, respectively. Harada et al. (2019), reported $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values for some of the samples collected in 2017 from the same study location.

The samples collected in 2017 showed substantial differences in $\delta^{13}\text{C}$ values between the two forests (Harada et al., 2019)
190 suggesting that they are representative of the dieback impact. The 2017 samples from each forest including the mangrove leaf (n=2), MPB (n=1), algae feeder (n=3), leaf feeder (n=2 to 3), grazer (n=3), filter feeder (n=2) were further measured for carbon isotopic composition of individual amino acids ($\delta^{13}\text{C}_{\text{AA}}$). ~~For this CSIA,~~ 8 mg (for animal tissues) or 30 mg (for plant tissues) of sample materials were transferred to borosilicate vials with heat and acid-resistant caps. They were then flushed with N_2 gas, sealed and hydrolysed in 0.5mL (animal tissues) or 2mL (plant tissues) of 6M HCl at 150°C for 70 minutes, then dried in
195 a heating block at 60°C under a stream of N_2 gas. The dried samples were derivatised by methoxycarbonylation as described by Walsh et al. (2014). Amino acid derivatives were separated by a Trace GC Ultra gas chromatograph (Thermo Scientific) using a DB-23 column, Agilent, 30m x 0.25mm, 0.25 μm film at the stable isotope facility at the University of California

(Davis, CA, USA). The GC was interfaced with a Delta V Plus isotope ratio mass spectrometer via a GC IsoLink (Thermo Scientific). L-Norleucine was used as an internal standard and to calculate provisional values.

200

Pure AAs mixtures with calibrated $\delta^{13}\text{C}$ were co-measured. One mixture was used for final calibration and others were for the scale normalisation standard and the primary quality assurance standard (unused in corrections). Two working standards were co-measured as secondary quality assurance materials. Exogenous carbon was accounted by the method detailed by Docherty et al. (2001). Following these processes, $\delta^{13}\text{C}$ values were determined for 10 AAs (Gly, glycine; Asx, aspartic acid/asparagine; Pro, proline; Glx, glutamic acid/glutamine; Ala, alanine; Lys, lysine; Ile, isoleucine; Leu, leucine; Phe, phenylalanine; and Val, valine). Met, methionine; His, histidine; and Hyp, hydroxyproline were at or below the limit of quantitation (LOQ) for some samples. Since we were interested in $\delta^{13}\text{C}$ values of essential amino acids ($\delta^{13}\text{C}_{\text{EAA}}$), ~~we only report~~ $\delta^{13}\text{C}$ values of Lys, Ile, Leu, Phe and Val were reported in the study.

205

2.4 Data analysis

210 All statistical analyses were undertaken in R version 3.4.3 with RStudio interface version 1.1.414. Differences among group means were explored with ANOVA, but for the count data i.e. seedling/ sampling populations, generalized linear model (GLM) with Poisson distribution was used. Before performing ANOVA, the assumptions of homogeneity of variance and normality were tested using Levene's and Shapiro-Wilk's tests, respectively. Two way ANOVA was used to test the effects of time (year) and forest type (unimpacted and impacted) on stable isotope values of invertebrates. To explore $\delta^{13}\text{C}$ patterns among five EAs, $\delta^{13}\text{C}_{\text{EAA}}$ values were normalised to the respective sample means following the procedure of Larsen et al. (2009) as follows:

215

$$\text{Norm}(\delta_{\text{EAA}}) = \delta_{\text{EAA}} - \mu \quad (1)$$

where μ represents the mean value of all five EAAs (Ile, Leu, Lys, Phe and Val) in the sample. PERMANOVA was performed to test if the pattern of $\delta^{13}\text{C}$ among five EAAs of samples differ between the forests. In this analysis, the normalized $\delta^{13}\text{C}_{\text{EAA}}$ dataset was used and the Euclidean distance was used as distance metric. Permutation test of multivariate homogeneity of dispersions was performed to check whether dispersions around the centroids are similar between the two forests. All statistical tests used a significance criterion of $\alpha=0.05$.

220

3 Results

3.1 Mangroves

225 In 2016, ~~approx. 8 months after the mangrove mortality event~~, at the impacted site, mangrove seedling and sapling populations were lower than at the unimpacted site (GLM df = 1, estimate 3.75, $p < 0.001$) but significantly increased throughout the period from 2016 to 2018 (GLM df = 2, estimate 0.01, $p < 0.001$; Fig. 2). C, N and S elemental compositions (%) of ~~the dominant mangrove species, A. marina~~, did not differ significantly greatly between the two sites, but the isotopic compositions varied

230 ~~significantly considerably in 2018 (32 months after the dieback;~~ Table 2). The $\delta^{13}\text{C}$ values (mean \pm SD) of green leaves harvested from *A. marina* trees, were significantly higher (i.e. more ^{13}C -enriched) in the impacted site ($-25.8 \pm 1.0\text{‰}$) than the unimpacted site ($-28.4 \pm 1.5\text{‰}$; ANOVA $F_{1, 28} = 32.9$, $p < 0.001$; Fig. 3A, Table 2). The $\delta^{15}\text{N}$ values varied more in the impacted site (ranged from -0.9 to 6.7‰) than in the unimpacted site (ranged from 2.9 to 6.2‰ ; Fig. 3A). The $\delta^{34}\text{S}$ values were generally higher in the impacted site ($13.5 \pm 5.4\text{‰}$, range 7.7 to 23.3‰) than the unimpacted site ($12.6 \pm 5.6\text{‰}$, range 5.0 to 21.9‰). Leaf $\delta^{34}\text{S}$ values became more ^{34}S depleted from higher to lower intertidal zones in the impacted site (ANOVA $F_{4, 10} = 5.56$, $p = 0.013$; Fig. 3B). This pattern was weaker in the unimpacted site, but leaf $\delta^{34}\text{S}$ values significantly substantially varied across intertidal zones (ANOVA $F_{4, 10} = 6.48$, $p = 0.007$; Fig. 3B). Leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values did not display such patterns along the intertidal zones (Table S2). Leaf C, N and S (%) did not show any clear trends among two forests and along transects. Yellow leaves generally had a higher S content ($\sim 1.2\%$) than green leaves (0.5 to 0.7%) (Table 2). Wood samples generally had very low N and S contents, significantly lower than the leaves. ~~However, wood samples from the impacted site~~ 240 ~~had a relatively high S content ($0.31 \pm 0.37\%$) and had a $\delta^{34}\text{S}$ value of 16.6‰ (Table 2).~~

3.2 Sediment

For the surface (~~≤ 0~~ – 0.5 cm) sediment collected in 2018 (~~32 months after the dieback~~), TOC (%) differed significantly between the two forests with the values (mean \pm SD) of $2.02 \pm 1.16\%$ for the unimpacted site and $1.06 \pm 0.37\%$ for the impacted site (ANOVA $F_{1, 28} = 12.75$, $P = 0.001$), suggesting that the surface sediment from the impacted site contains $\sim 48\%$ lower TOC 245 relative to those of the unimpacted site (Table 3). ~~The pattern was consistent across the intertidal zones (Fig 4A).~~ The surface sediment TN (%) was also significantly lower for the impacted site (0.09 ± 0.03) than the unimpacted site ($0.15 \pm 0.06\%$; ANOVA $F_{1, 28} = 9.32$, $P = 0.005$). TOC of mudflat (< 0.5 cm) sediment collected adjacent to the two forests also differed significantly with those of impacted site being lower ($0.58 \pm 0.18\%$) than the unimpacted site ($1.02 \pm 0.08\%$; ANOVA $F_{1, 4} = 14.54$, $P = 0.019$). TN (%) of mudflat (< 0.5 cm) sediment was also significantly lower for the impacted site (ANOVA $F_{1, 4} = 250 9.81$, $P = 0.035$). TOC (%) of $0.5 - 20$ cm sediment did not differ significantly between the two sites with the values of $1.83 \pm 0.73\%$ for the unimpacted and $1.29 \pm 0.55\%$ for the impacted site (ANOVA $F_{1, 10} = 2.07$, $P = 0.181$). TN (%) of $0.5 - 20$ cm sediment also did not differ significantly between the two sites with the values of $0.09 \pm 0.03\%$ for the unimpacted and $0.12 \pm 0.04\%$ for the impacted site (ANOVA $F_{1, 10} = 3.02$, $P = 0.113$). Despite the substantial variation in TOC and TN, the C/N ratio did not differ significantly between the two sites (ANOVA $P > 0.05$).

255 The $\delta^{13}\text{C}$ values of surface (~~< 0.5 cm~~) sediment differed significantly between the two sites with those from the impacted site showing higher values ($-21.8 \pm 1.0\text{‰}$) than those from the unimpacted site ($-24.3 \pm 1.2\text{‰}$; ANOVA $F_{1, 28} = 22.48$, $P < 0.001$). This pattern was consistent across the intertidal zones with $\delta^{13}\text{C}$ values from the impacted site becoming similar to those from the adjacent mudflat (Fig. 4; Table S3b). However, those of 0.5 to 20 cm sediment did not differ significantly with the values 260 of $-24.4 \pm 0.5\text{‰}$ for the impacted site and $-25.2 \pm 0.9\text{‰}$ for the unimpacted site (ANOVA $F_{1, 10} = 3.92$, $P = 0.076$). Surface (~~$<$~~

265 ~~0.5 cm~~ sediment collected in the adjacent mudflat did not display a significant difference in $\delta^{13}\text{C}$ values between the two sites with the values of $-21.2 \pm 0.9\text{‰}$ for those collected adjacent to the unimpacted forest and $-21.8 \pm 0.8\text{‰}$ for those collected adjacent to the impacted site (ANOVA $F_{1,4} = 0.64$, $P = 0.47$). The $\delta^{13}\text{C}$ value of the surface sediment ($-21.8 \pm 1.0\text{‰}$) in the impacted forest was similar to the value of those collected in the mudflat ($-21.2 \pm 0.9\text{‰}$) which were also similar to those collected from offshore ($-21.58 \pm 1.1\text{‰}$). Those $\delta^{13}\text{C}$ values also matched with the $\delta^{13}\text{C}$ value of POM collected offshore ($-21.5 \pm 1.5\text{‰}$). Surface ~~(<0.5 cm)~~ sediment collected from adjacent unvegetated saltpan areas also showed similar values (Table 3). MPB extracted from the surface ~~<0.5 cm~~ sediment showed significantly different $\delta^{13}\text{C}$ values between the impacted site ($-21.5 \pm 1.3\text{‰}$) and unimpacted site ($-25.2 \pm 1.0\text{‰}$; ANOVA $F_{1,10} = 28.53$, $P < 0.001$).

3.3 Fauna

270 CNS isotopic compositions of mangrove macroinvertebrates representing algae feeder ~~algivores~~, grazers and leaf feeders from the impacted site were ~~consistently~~ more enriched in ^{13}C , ^{15}N and ^{34}S than their counterparts from the unimpacted forest throughout the period between 2016 and 2018 (Fig. 5). However, the filter feeding oyster that relies on water column organic matter showed relatively less differences between the two forests (Fig. 5). Overall, $\delta^{13}\text{C}$ values of the four feeding groups range from -23 to -15‰ for the unimpacted site and -20 to -14‰ for the impacted site. The $\delta^{15}\text{N}$ values ranged from 5.5 to
275 9.1‰ for the unimpacted site with the fauna in the impacted site having a slightly higher range of 5.6 to 9.5‰ . The $\delta^{34}\text{S}$ values ranged from 8.2 to 16‰ for the unimpacted site and 13.4 to 21.7‰ for the impacted site. ~~The~~ The effect of forest type was significant for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of the algae feeder and the grazer significantly differed between the two forests (ANOVA $p < 0.05$). ~~The~~ The effect of forest type was also significant for $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values of the leaf feeder significantly differed between the two forests (ANOVA $p < 0.05$), but ~~was not significant for~~ the $\delta^{15}\text{N}$ values were not significantly different
280 (ANOVA $F_{1,13} = 1.72$, $p = 0.212$). ~~The effect of forest type was not significant for~~ the filter feeder $\delta^{13}\text{C}$ values did not differ between the two forests (ANOVA $F_{1,8} = 1.719$, $p = 0.212$), but ~~was significant for~~ the $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values differed significantly between the two forests (ANOVA $p < 0.05$). The effect of time was significant for the leaf feeder $\delta^{34}\text{S}$ values, the grazer $\delta^{15}\text{N}$ values, the filter feeder $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values (ANOVA $p < 0.05$). ~~Overall, the $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ values of mangrove invertebrates consistently differed between the sites during the period from 2016 to 2018.~~ In 2018, leaf feeder $\delta^{13}\text{C}$
285 values, grazer $\delta^{34}\text{S}$ values and algae feeder $\delta^{34}\text{S}$ values significantly differed between the impacted and unimpacted sites (Turkey post hoc test, $P < 0.05$), showing no recovery of the invertebrate fauna $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ status ~~after~~ 32 months after from the dieback event. However, $\delta^{15}\text{N}$ values ~~started showing matches~~ became similar between the two forests in 2018 (Fig. 5).

3.4 Compound-specific isotope analysis of amino acid carbon

The samples collected in 2017 were further measured for carbon isotopic compositions in individual essential amino acids.
290 $\delta^{13}\text{C}_{\text{EAA}}$ values corresponded to the bulk $\delta^{13}\text{C}$ values with the samples from the impacted site consistently showing higher values than those from the unimpacted forest (Table 4 and Fig 6). The pattern of normalized $\delta^{13}\text{C}$ among five EAAs (Lys, Ile,

Val, Leu and Phe) for all the consumers did not differ between the two ~~forests forest types~~ ($p > 0.05$; Table 4 and Fig S1); ~~including the algae feeder, leaf feeder, grazer and filter feeder~~. The $\delta^{13}\text{C}_{\text{EAA}}$ pattern of mangrove leaves did not differ between the forests, regardless of the substantial bulk isotope difference between the unimpacted ($-26.7 \pm 2.2\%$) and impacted ($-25.4 \pm 0.1\%$) (Table 4). This isotope pattern was similar for all four feeding groups as well as for the MPB samples (Fig. 6 and Fig S1).

4. Discussion

4.1 Mangroves

The recovery of mangrove forests from tree mortality events caused by disturbances such as cyclones, generally relies upon the recruitment of seedlings (Smith et al., 1994; Krauss and Osland, 2019). Subsequently, degraded habitats with a reduced seed pool, production and delivery, e.g. by habitat fragmentation, may show slower forest recovery (Milbrandt et al., 2006). The establishment of seedlings may also be inhibited by persistent inundation due to a decreased sediment elevation (Cahoon et al., 2003; Asbridge et al., 2018). ~~M~~Although mangroves are resilient ecosystems and may recover quickly from natural disturbances (Sherman et al., 2001), ~~but~~ in some cases, ~~full~~ recovery may take ~~decades more than 10 years~~ (Imbert et al., 2000; ~~Connolly et al., 2020~~). Further, mangrove degradation may be followed by fast colonisation of non-mangrove herbaceous species, ~~e.g. succulent saltmarsh (Mbense et al., 2016; McKee et al., 2007; Rashid et al., 2009), e.g. succulent saltmarsh (Mbense et al., 2016). However, this was not largely evident from our study site. In both mangrove forests at the impacted Gulf of Carpentaria site mangrove forest, the density of mangrove seedling/samplings significantly increased throughout the period from 2016 to 2018, suggesting that recovery was starting to occur in some areas within 32 months after the dieback and propagule pool was available in the vicinity. The increase in the seedling/sampling density at the unimpacted site was unexpected, but this indicates that there was some stress at the unimpacted site during the dieback period and/or the temporal variability of seedling/samplings density was high at the site (Fig. 2).~~

The substantial differences in CNS isotopic compositions in *A. marina* occurring between the two sites, suggested differences in the environmental conditions and biogeochemical processes that were possibly associated with the mangrove mortality effect. The leaf $\delta^{13}\text{C}$ values in the impacted forest were relatively enriched in ^{13}C . This C isotope pattern may be due to reduced stomatal conductance that causes lower internal carbon dioxide concentrations and lower carbon isotope fractionation (Farquhar et al., 1989; Lin and Sternberg, 1992a; Lin and Sternberg, 1992b). ~~Higher ^{13}C -enriched~~ leaf $\delta^{13}\text{C}$ values can also be associated with increased carboxylation efficiency associated with higher nutrients, e.g. N in leaves (Cordell et al., 1999) and thicker leaves with higher internal resistance to carbon dioxide diffusion. Younger leaves can show higher $\delta^{13}\text{C}$ values than aged leaves due to ^{13}C enriched fractions (e.g. carbohydrates) transported from older autotrophic leaves to more heterotrophic young leaves (Werth et al., 2015). Leaves exposed to full sun can show higher $\delta^{13}\text{C}$ values than shaded leaves (Farquhar et al., 1989). $\delta^{13}\text{C}$ values can also vary among different plant tissues in *A. marina* (Kelleway et al., 2018). Leaf N (%) did not differ

325 between the two forests, suggesting that the two sites may have similar plant N availability. Previous studies show additions of nutrients such as N and/or P did not play a considerable role in mangrove leaf $\delta^{13}\text{C}$ variations (McKee et al., 2002), but salinity played an important role (Lin and Sternberg, 1992b). For example, leaf $\delta^{13}\text{C}$ values of *A. marina* at a lower salinity site was relatively depleted in ^{13}C and averaged about -31‰ (Kelleway et al., 2018). Overall, ~~higher ^{13}C -enriched~~ leaf $\delta^{13}\text{C}$ values in the impacted forest likely suggest that there are chronic stresses associated with the dieback event that reduced stomatal conductance. Such environmental stresses may include hypersalinization of sediments and hydric, thermal and radiant stresses following ~~mangrove losses (e.g. canopy losses that cause higher evaporation and lower water availability)~~. The leaves at the unimpacted site were largely depleted in ^{13}C , suggesting that there was higher water availability at the unimpacted site, possibly associated with regional variability in groundwater flows, e.g., Sippo et al. 2020.

335 Leaf $\delta^{15}\text{N}$ values varied more in the impacted (ranged from -0.9 to 6.7‰) than in the unimpacted forest (ranged from 2.9 to 6.2‰) (~~Fig. 3~~), but the means were similar ($4.3 \pm 1.9\text{‰}$ for the impacted and $4.4 \pm 0.8\text{‰}$ for the unimpacted), suggesting that two sites have similar background $\delta^{15}\text{N}$ conditions. Generally, leaf $\delta^{15}\text{N}$ varies due to N sources, microbial processes that enrich or deplete ^{15}N in soil or water, and isotope fractionation during plant N uptake. Previous studies showed that in pristine mangrove forests, leaf $\delta^{15}\text{N}$ values generally range around -2‰ to 3‰ (Fry and Smith, 2002; Smallwood et al., 2003). Such low $\delta^{15}\text{N}$ values may reflect long-term N fixation inputs (e.g. around 0‰) (Fogel et al., 2008) and marine nitrate inputs (Dore et al., 2002). Much higher $\delta^{15}\text{N}$ values (>10‰) may be associated with anthropogenic N inputs (Fry and Cormier, 2011). Our sites showed moderate $\delta^{15}\text{N}$ values (about 4‰), suggesting that in addition to N fixation inputs and marine N inputs, there may be considerable microbial ^{15}N enrichment in dissolved inorganic nitrogen pools of ammonium and nitrate. The higher variability in leaf $\delta^{15}\text{N}$ in the impacted forest suggests higher variability in processes affecting the $\delta^{15}\text{N}$ status of available N. For example, changes to sediment conditions including redox transitions and soil moisture content following the dieback may 345 have affected microbial processes of N, whereas the unimpacted forest may have had more stable sediment conditions and N pool, ~~processes and inputs~~. Isotope fractionation during plant N uptake may also be an explanation for leaf $\delta^{15}\text{N}$ variability (Fry et al., 2000), but such fractionation is poorly known for mangroves. A study reported that additions of P nutrients increased N demand and decreased ^{15}N fractionation (McKee et al., 2002), however as we did not measure P, we could not determine whether this was the case.

350 Leaf $\delta^{34}\text{S}$ values differed considerably between the two forests, with the impacted forest generally having higher values ($13.5 \pm 5.4\text{‰}$, range 7.7 to 23.3‰) than the unimpacted forest ($12.6 \pm 5.6\text{‰}$, range 5.0 to 21.9‰). Leaf $\delta^{34}\text{S}$ values showed trends along the six transects, with values decreasing from the upper to lower intertidal zones (~~Fig. 3B~~). Based on previous studies, mangrove leaf $\delta^{34}\text{S}$ values generally vary between -20 to 20‰ (Okada and Sasaki, 1995, 1998; Fry and Smith, 2002). Higher 355 $\delta^{34}\text{S}$ values are likely associated with seawater sulfate, which is ^{34}S enriched (i.e. 21‰) and due to a large isotope fractionation (up to 70‰) during sulfate reduction (Kaplan and Rittenberg, 1964). Lower $\delta^{34}\text{S}$ values are likely associated with sedimentary

sulfide-S that is ^{34}S depleted, for example, -21‰ (Okada and Sasaki, 1995). Leaf $\delta^{34}\text{S}$ values of around 14 to 18‰ suggest mangrove incorporations of seawater sulfate-S ($\delta^{34}\text{S}$, ~ 21‰), with only a small isotopic fractionation occurring through absorption and assimilation steps (Okada and Sasaki, 1995). Plants generally show $\delta^{34}\text{S}$ values slightly lower than source sulfate-S by an average of -1.5‰ (Trust and Fry, 1992). Low leaf $\delta^{34}\text{S}$ values, for instance, the lowest value of 5‰ found in the unimpacted site –suggest that the most probable source of this ^{34}S -depleted S is sulfide oxidation, followed by mixing with seawater sulfate. –Low $\delta^{34}\text{S}$ values in mangrove root vascular tissues may indicate assimilation/oxidation of sulfide, potentially to reduce their toxic sulfide exposure (Fry et al., 1982; Raven et al., 2019), with reported isotope effect of -5.2‰ for non-biological oxidation of sulfide (Fry et al., 1988) and a smaller +1-3 ‰ effect for anaerobic oxidation of sulfide by photosynthetic bacteria (Fry et al., 1984).

An explanation for our observed $\delta^{34}\text{S}$ pattern may be lower plant incorporation of sulfide-S in the impacted site and also in the higher intertidal zones where we expect that mangrove sediment is relatively more oxidised, and the production of sulfide may be lower due to lower sulfate reduction. ~~High wood $\delta^{34}\text{S}$ values (16.6‰) and S content (0.31%) in the impacted forest may suggest degradation of wood by fungi and/or bacteria that incorporate seawater sulfate-S and increase overall wood $\delta^{34}\text{S}$ values and S content. Such $\delta^{34}\text{S}$ patterns have been reported in mangroves (Fry and Smith, 2002) and saltmarsh (Currin et al., 1995), where $\delta^{34}\text{S}$ values of fresh organic matter evolved during degradation steps and gradually increased towards the $\delta^{34}\text{S}$ value of seawater sulfate S (i.e. 21‰).~~

4.2 Sediment

In healthy mangrove forests, the fate of C fixed by primary producers includes burial within the sediment, atmospheric emissions and outwelling to the ocean (Maher et al., 2018), but how mangrove mortality affects such processes is poorly understood. In most cases, C within in mangrove sediment decreases following forest loss due to degradation with increased CO_2 emissions (Otero et al., 2017; Adame et al., 2018). –Lower TOC (%) and higher sediment $\delta^{13}\text{C}$ values in the impacted forest (Table 3 and Fig. 4) are probably related to sediment C loss and lower autochthonous mangrove-C inputs (i.e. leaf litter) following the mangrove mortality event. Consistent with this, the sediment N (%) and $\delta^{15}\text{N}$ data showed a similar pattern suggesting N loss and degradation. The surface sediment (0–0.5 cm) ~~varied/differed relatively~~ more than the subsurface/deeper (0.5 to 20 cm) fraction. ~~One explanation for this is probably because that~~ the surface ~~sediment~~ fraction is generally more aerobic, and ~~therefore~~ remineralization of organic matter occurs more rapidly (Burdige, 2011). Sediment $\delta^{13}\text{C}$ and ^{15}N values can increase during degradation of sediment organic matter following mangrove loss (Adame and Fry, 2016;). ~~This isotope pattern has been reported following mangrove loss~~ (Adame et al., 2018). Changes in sediment C and N may also be associated with root turnover. The MPB $\delta^{13}\text{C}$ values significantly differed, with those from the impacted being higher ($-21.5 \pm 1.3\text{‰}$) than the unimpacted ($-25.2 \pm 1.0\text{‰}$). The higher values probably indicate lower respiratory inputs of CO_2 from mangroves (Maher et al., 2013b). Our findings here are consistent with the finding of Sippo et al. (2019) that changes to oceanic carbon

390 outwelling rates following mangrove loss are likely associated with a gradual loss of sediment carbon; similar to our finding of increased sediment $\delta^{13}\text{C}$ values in the impacted site, an isotope effect may have been due to loss of sediment mangrove C and/or replacement of mangrove peats with marine sediment.

4.3 Fauna

CNS isotopic compositions of consumers including an algae feeder, a grazer and a leaf feeder from the impacted site were ~~consistently~~ more enriched in ^{13}C , ^{15}N and ^{34}S . These differences ~~remained consistent did not change~~ throughout the three 395 samplings of 2016, 2017 and 2018 ~~(Fig. 5)~~. Consistent with the findings from mangrove leaves, MPB and soil, ~~these~~ data suggested substantial changes in cycling of CNS associated with the mangrove mortality event. ~~Overall, the consumer $\delta^{13}\text{C}$ values ranged from -22.9 to -15.2‰ for the unimpacted site, but at~~ the impacted site, consumers were more ^{13}C -enriched, ~~(range of -20.0 to -14.0‰)~~, likely due to the loss of ^{13}C -depleted mangrove organic matter. Consumer $\delta^{13}\text{C}$ values can change due to changes to available organic matter, altered feeding dependencies as well as changes to organic matter $\delta^{13}\text{C}$ values. In 400 our case, For example, MPB $\delta^{13}\text{C}$ values can change significantly, likely due to changes to organic matter respiratory inputs and/or altered light environment and soil moisture contents that may change isotopic fractionation during carbon fixation in response to organic matter respiratory inputs. The consumer $\delta^{13}\text{C}$ values and their ranges at our study site are fairly consistent with the reported mangrove consumer $\delta^{13}\text{C}$ values elsewhere (e.g. Lee, 2000; Bouillon et al., 2002; Demopoulos et al., 2007). The typical trophic enrichment factor for carbon isotope in small invertebrates is about +1‰ (Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003). Lower consumer $\delta^{13}\text{C}$ values ~~near -27‰~~ are generally associated with mangrove detritus that is depleted in ^{13}C ., ~~but in many cases,~~ Typical mangrove leaf-eating sesarimid crab species (i.e. Sesarimidae) generally have tissue $\delta^{13}\text{C}$ values can be enriched within by about $\pm 5\%$ from the mangrove detritus (Bui and Lee, 2014). Higher $\delta^{13}\text{C}$ values of consumers are generally tied to MPB, ~~with a typical isotope effect during assimilation, e.g. $< -1\%$ estimated for small invertebrates (Vander Zanden and Rasmussen, 2001; McCutchan et al., 2003).~~ Our MPB endmember $\delta^{13}\text{C}$ values of - 410 25.2‰ for the unimpacted site and -21.5‰ for the impacted site did not match with the consumer $\delta^{13}\text{C}$ values (around -15 to -14‰), suggesting our characterization of MPB endmember $\delta^{13}\text{C}$ values was incomplete. This is probably because MPB can vary substantially within mangrove ecosystems (Bouillon et al., 2008) and consumers may be preferentially assimilating more ^{13}C enriched fractions of MPB, for example, diatom and/or filamentous cyanobacteria that can range about -15 to -20‰ (Craig, 1953; Fry and Wainright, 1991). The leaf feeders ~~in this study were relatively depleted in ^{13}C showed with~~ $\delta^{13}\text{C}$ values of 415 about -21 to -18‰, ~~and was substantially enriched compared to mangrove leaves (-27 to -25‰), consistent with the findings of Bui and Lee (2014). This also likely due to~~ indicated some use of mangrove leaves, additional contributions from other sources such as MPB.

Due to difficulties obtaining representative endmembers, mixing analysis ~~using sampled organic matter~~ was not achieved ~~in~~ 420 ~~this using this data study~~ to quantify feeding dependencies. However, MPB probably played an important dietary role in the

both forests because the difference in MPB $\delta^{13}\text{C}$ values between the two forests were reflected in the difference in the consumers $\delta^{13}\text{C}$ values between the two forests (Harada et al. 2019). ~~Alternatively, the consumer data was used to help infer endmembers and assess feeding dependencies, e.g. Riekenberg et al. (2016). POM (-21.1‰) matched with the filter feeders and seemed to be an important food source for the all consumers in both forests. Mangrove leaves (-27 to -25‰) did not seem to be an important source for the consumers with the lowest consumer being -22.9‰ at the unimpacted site and -20.0‰ at the impacted site. The consumers were generally higher than the POM with the highest consumer being -15.2‰ at the unimpacted site and -14.0‰ at the impacted site, suggesting that there was a substantial contribution from more ^{13}C enriched MPB.~~

Consistent with the mangrove leaf $\delta^{34}\text{S}$ values, the consumer $\delta^{34}\text{S}$ values also indicated possible changes to S cycling. The consumer $\delta^{34}\text{S}$ values were generally higher in the impacted site (range 13.4 to 21.7‰) than in the unimpacted site (range 8.2 to 16‰) suggesting lower sulfate reduction with decreased sulfide inputs at the impacted site. Fixation of sulfate by phytoplankton occurs with a small isotope effect, around 1 to 2‰ (Fry, 2006), therefore, ~~phytoplankton $\delta^{34}\text{S}$ values from the coastal ocean are generally close to the seawater sulfate-S value of 21‰, so that $\delta^{34}\text{S}$ values of phytoplankton from the coastal ocean should be close to the seawater sulfate-S value of 21‰.~~ MPB generally have lower $\delta^{34}\text{S}$ values than phytoplankton, e.g. with reported average values near 10‰ for MPB in a mangrove ecosystem (Harada et al., unpublished), likely due to some use of sedimentary sulfide-S (depleted in ^{34}S). Our ~~consumer mangrove leaf $\delta^{34}\text{S}$ values were lower than 21‰, suggesting some use of MPB as well as mangrove detritus, averaged 13.5‰ for the impacted site and 12.6‰ for the unimpacted site, lower than the seawater sulfate S. For these reasons, the unimpacted site that had lower consumer $\delta^{34}\text{S}$ values could be associated with sulfide inputs with some use of mangrove organic matter and MPB, whereas the impacted site that had higher consumer $\delta^{34}\text{S}$ values are associated more with seawater sulfate. This indicates a change to the S cycling and use of S by plants as well as microbial intermediates in the food web.~~

The consumer $\delta^{15}\text{N}$ also indicates possible changes to N cycling, with the consumer in the impacted site generally having higher values than those from in the unimpacted site. The higher $\delta^{15}\text{N}$ ~~values are likely may be~~ associated with degradation of organic matter, ~~microbial ^{15}N enrichment in dissolved inorganic N such as ammonium and nitrate during degradation and degradation, and ^{15}N enrichment by microbial intermediates in the food web. The high ^{15}N may also indicate~~ lower N fixation inputs that typically show low $\delta^{15}\text{N}$ values, ~~round 0‰.~~ While the $\delta^{13}\text{C}$ and $\delta^{34}\text{S}$ values consistently differed between the two forests during the two-year survey, the $\delta^{15}\text{N}$ values started showing matches between the two forests in 2018, ~~likely. This may~~ suggesting recovery of $\delta^{15}\text{N}$ status to the background conditions, and/or that the recovery of N may be faster than C and S elements. This may be the case as mangrove ecosystems are generally N limited (Reef et al., 2010), and circulation of N elements is faster than those of C and S elements.

4.4 Compound-specific isotope analysis of amino acids

455 It is considered that environmental resources such as vascular plants and microalgae have a different $\delta^{13}\text{C}$ pattern ('fingerprint')
in AAs due to differing biosynthesis of AAs (Larsen et al., 2009; Larsen et al., 2013). It is also reported that $\delta^{13}\text{C}$ patterns are
largely unaffected by environmental conditions. For example, $\delta^{13}\text{C}_{\text{AA}}$ patterns of the marine diatom *Thalassiosira weissflogii*
did not respond to changing environmental conditions such as light, salinity, temperature and pH, despite substantial changes
in bulk $\delta^{13}\text{C}$ values (Larsen et al., 2015). A similar isotope pattern was reported for seagrass *Posidonia oceanica* and the giant
460 kelp *Macrocystis pyrifera*, which showed consistent $\delta^{13}\text{C}_{\text{AA}}$ patterns despite varying season and growth conditions (Larsen et
al., 2013). The $\delta^{13}\text{C}_{\text{AA}}$ patterns in producers, especially those of essential amino acids ($\delta^{13}\text{C}_{\text{EAA}}$), can be ean-reflected in
consumer tissues with little isotope effect. This is ~~o~~ccurs because animals obtain EAAs from their diet and EAA fractions are
thought to be directly assimilated (McMahon et al., 2010). These general expectations were reasonably met in our $\delta^{13}\text{C}_{\text{EAA}}$
dataset ~~—that was normalized to means of five EAAs as per Larsen et al. (2009)~~. Normalized $\delta^{13}\text{C}_{\text{EAA}}$ patterns of our producer
465 samples including mangrove leaves (yellow leaves of *A. marina*) and MPB did not differ between the two sites despite differing
environmental conditions and substantial differences in bulk $\delta^{13}\text{C}$ values (Table 4, Fig 6 and Fig S1). Furthermore, the
consumer $\delta^{13}\text{C}_{\text{EAA}}$ patterns also did not differ between the two sites. (Fig. 6 and Fig S1). These findings did not support changes
to feeding dependency following mangrove loss but suggested that the overall differences in the consumer bulk $\delta^{13}\text{C}$ values
were most likely driven by differences in the resource organic matter $\delta^{13}\text{C}$ values e.g. changes to MPB $\delta^{13}\text{C}$ values that were
470 likely associated with lower mangrove C fixation/respiratory inputs following the mangrove mortality. Furthermore, such
findings indicate that the reported substantial change to the mangrove benthic faunal assemblage following the mangrove loss
(Harada et al., 2019) was probably driven more by modification of physical habitat structure than changes in the use of food
resources.

5 Conclusions

475 Reporting rare and extreme biological events can be complicated because in many cases they may occur suddenly, therefore
drawing comparisons between pre and post event conditions remains a challenge. Our field investigations using traditional
ecological techniques combined with SIA ~~—~~measured the initial dieback and also early recovery of an impacted mangrove
ecosystem and compared an adjacent unimpacted reference system. Although the unimpacted and impacted forests were not
replicated in this study, the difference between the two sites ~~were~~as clear. Mangrove seedling and sapling populations that
480 increased during the period from 2016 to 2018 (8 to 32 months after the mortality event) in the impacted site, suggest recovery
of the mangrove vegetation. This also suggests that the environmental conditions at the impacted site are still conducive for
re-establishment of mangroves, allowing recruitment of seedlings and development of regrowth. However, mangrove leaves
collected in the impacted site in 2018 showed relatively higher $\delta^{13}\text{C}$ values ($-25.8 \pm 1.0\%$) that are probably associated with
continued water stress. Invertebrates from the impacted site representing the feeding types of grazing, leaf feeding, and algae

485 feeding were more enriched in ^{13}C , ^{15}N and ^{34}S relative to those from the unimpacted site. For example, they were more ^{13}C
enriched at the impacted site, by 1.7 – 4.1‰ and the difference did not change over the study period. Overall, our stable CNS
isotope data supported the hypothesis that changes to biogeochemical processes occur following the mangrove mortality. These
changes include lower mangrove C fixation/respiration, lower N fixation and lower sulfate reduction. However, our isotope
data did not support the second hypothesis that the isotopic compositions change over time with recovery of mangrove
490 vegetation. Recovery of biogeochemical processes was not evident even after two years, suggesting an ongoing impact of the
mortality event. An exception was that N cycle recovery may be occurring faster.

Considering that the environmental conditions at the site play an important role in facilitating recolonisation of mangroves, we
conceptualise the recovery of the mangrove forest under four different scenarios to give insight into the ecological and
495 biogeochemical consequences of changing forest conditions (Fig. 7): 1) The forest recovers with mangroves being able to
recolonise at the site without future perturbations; 2) the forest recovers with future perturbations such as climatic events, for
example, mangrove recolonisation is driven by events as such ENSO cycles; 3) the forest does not recover and is transformed
into intertidal mudflats; and 4) the forest recovers partially at the site and in a reduced size and/or is recolonised by other plants
such as saltmarshes, e.g., mangroves only recolonise in the lower intertidal zone. Each of these scenarios will have a distinct
500 isotopic trajectory for C, N and S. While these are likely scenarios and there might be other possibilities, comparing the
impacted forest and an adjacent unimpacted reference forest can help us quantify the recovery. Continued monitoring of the
post-dieback forest would be required to predict the long-term trajectory of ecosystem recovery and how on-going climate
change and extreme climatic events affect the recovery of mangroves in the impacted region. In such a long-term investigation,
SIA is a powerful tool, capable of tracking changes in biogeochemical processes over time. As such, it is of great assistance
505 in ecosystem analyses and detecting the underlying biological mechanisms that drive changes and recovery.

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715 **Table 1.** Spatial and temporal details of the sampling design (x = sampled).

	<u>Sampling plots along each transect</u>						<u>Year</u>		
	<u>Forest edge, landward</u>	<u>High</u>	<u>Mid</u>	<u>Low</u>	<u>Forest edge, seaward</u>	<u>Mudflat</u>	<u>2016, 8 months after dieback</u>	<u>2017, 20 months after dieback</u>	<u>2018, 32 months after dieback</u>
<u>Mangrove seedling count</u>			x				x	x	x
<u>Bulk SIA invertebrates*</u>		x	x	x			x	x	x
<u>CSIA invertebrates*</u>		x	x	x				x	
<u>Bulk SIA mangrove leaves</u>	x	x	x	x	x				x
<u>Bulk SIA mangrove wood</u>			x						x
<u>Bulk SIA surface sediment</u>	x	x	x	x	x	x			x
<u>Bulk SIA subsurface sediment</u>			x						x

*for invertebrate SIA, to gain sufficient sampling size, high, mid and low plots were pooled for analysis.

Analyses conducted for samples from each field campaign (x = analysed).

<u>Analysis</u>	<u>2016 (8 months after dieback)</u>	<u>2017 (20 months after dieback)</u>	<u>2018 (32 months after dieback)</u>
<u>Mangrove seedling count</u>	x	x	x
<u>Bulk SIA invertebrates</u>	x	x	x
<u>Bulk SIA mangrove</u>			x
<u>Bulk SIA sediment</u>			x
<u>CSIA invertebrates</u>		x	

720 **Table 2.** Elemental and isotopic compositions of the mangrove *A. marina* (mean, SD) in 2018, 32 months after the dieback.

Tissue type	Forest	%C	%N	%S	$\delta^{13}\text{C}$, ‰	$\delta^{15}\text{N}$, ‰	$\delta^{34}\text{S}$, ‰	n
Green leaf	Unimpacted	39.3 \pm 1.7	1.75 \pm 0.37	0.54 \pm 0.23	-28.4 \pm 1.5	4.4 \pm 0.8	12.6 \pm 5.6	15
	Impacted	40.0 \pm 1.6	1.82 \pm 0.45	0.73 \pm 0.14	-25.8 \pm 1.0	4.3 \pm 1.9	13.5 \pm 5.4	15
Yellow leaf	Unimpacted	41.6 \pm 2.2	0.67 \pm 0.23	1.18 \pm 0.47	-26.4 \pm 1.3	6.5 \pm 1.1	14.6 \pm 7.7	3
	Impacted	41.9 \pm 1.0	0.52 \pm 0.05	1.18 \pm 0.38	-26.2 \pm 0.6	7.4 \pm 0.2	12.5 \pm 2.8	3
Wood	Unimpacted	42.6 \pm 3.0	0.38 \pm 0.27	0.04 \pm 0.01	-24.8 \pm 1.8	6.1 \pm 2.0	-	2
	Impacted	39.9 \pm 5.7	0.40 \pm 0.16	0.31 \pm 0.37	-24.9 \pm 1.0	4.7 \pm 1.0	-16.6	2

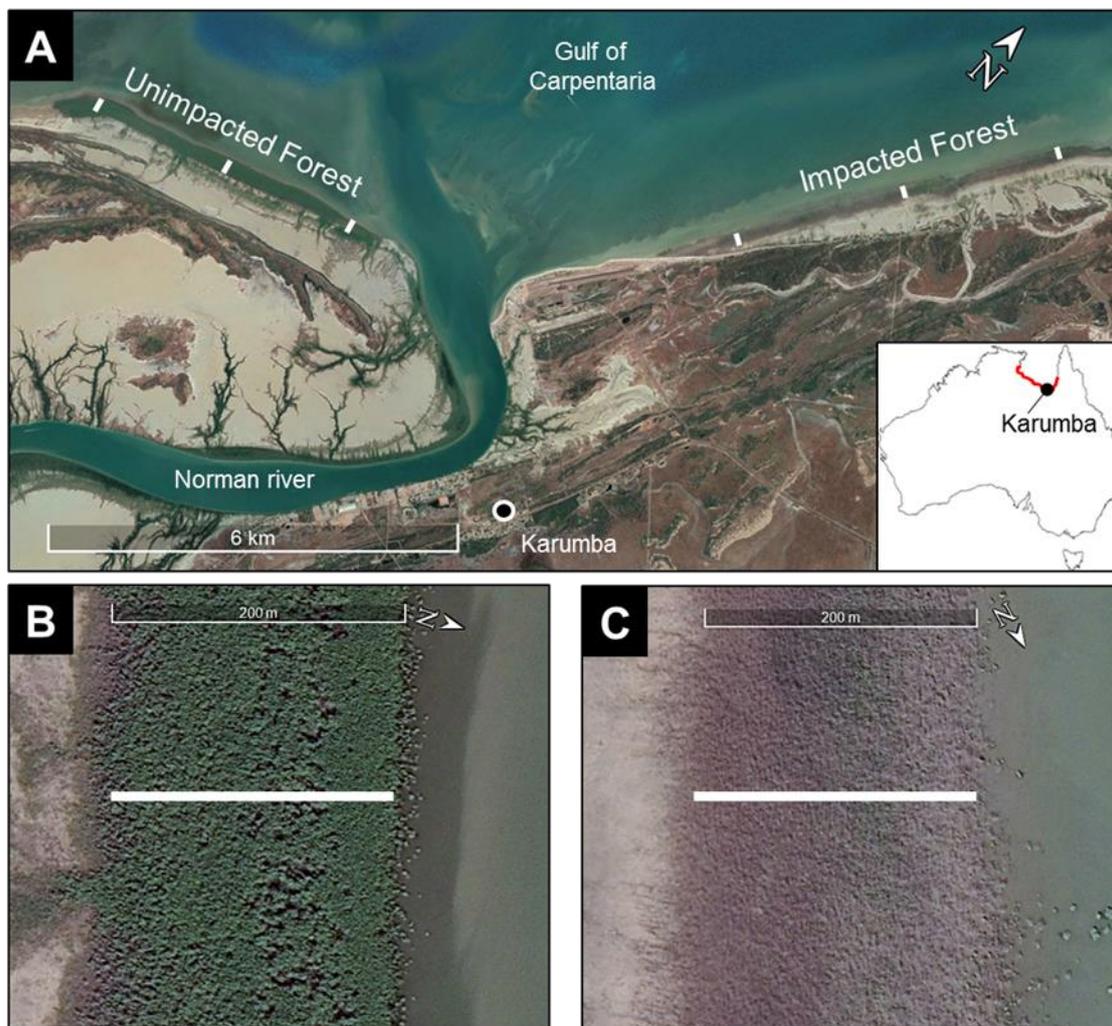
Table 3. Elemental and isotopic compositions of sediment in 2018, 32 months after the dieback (mean, SD).

	Forest/site	%TOC	%TN	C:N	$\delta^{13}\text{C}$, ‰	$\delta^{15}\text{N}$, ‰	n
Mangrove forest, 0 to 0.5 cm	Unimpacted	2.02 \pm 0.15	0.15 \pm 0.01	13.48 \pm 2.49	-24.3 \pm 1.2	2.0 \pm 0.5	15
		1.16	0.06			0.5*	
	Impacted	1.06 \pm 0.37	0.09 \pm 0.03	11.67 \pm 2.58	-21.8 \pm 1.0	2.8 \pm 0.6	15
		0.37	0.03			0.6*	
Mangrove forest, 0.5 to 20 cm	Unimpacted	1.83 \pm 0.73	0.12 \pm 0.04	15.12 \pm 1.79	-25.2 \pm 0.9	1.7 \pm 0.5	6
		0.73	0.04			0.5*	
	Impacted	1.29 \pm 0.55	0.09 \pm 0.03	14.71 \pm 2.81	-24.4 \pm 0.5	1.7 \pm 0.3	6
		0.55	0.03			0.3*	
Mudflat, 0 to 0.5 cm	Unimpacted	1.02 \pm 0.08	0.11 \pm 0.01	9.07 \pm 0.91	-21.8 \pm 0.8	-	3
		0.08	0.01				
	Impacted	0.58 \pm 0.18	0.07 \pm 0.02	8.78 \pm 0.93	-21.2 \pm 0.9	-	3
		0.18	0.02				
Saltpan, 0 to 0.5 cm	Unimpacted	1.87 \pm 2.03	0.19 \pm 0.24	11.44 \pm 3.05	-18.9 \pm 1.7	-	4
		2.03	0.24				
	Impacted	0.83 \pm 0.07	0.07 \pm 0.01	11.82 \pm 0.69	-20.8 \pm 0.7	-	4
		0.07	0.01				
Offshore, 0 to 0.5 cm	1km offshore	0.77 \pm 0.28	0.08 \pm 0.03	11.89 \pm 8.31	-21.58 \pm 1.1	-	5
		0.28	0.03		1.1		
POM	1km offshore	-	-	-	-21.1 \pm 1.5	3.6 \pm 2.1	3
MPB	Unimpacted	-	-	-	-25.2 \pm 1.0	-	6
	Impacted	-	-	-	-21.5 \pm 1.3	-	6

*values were taken from Harada et al. (2019)

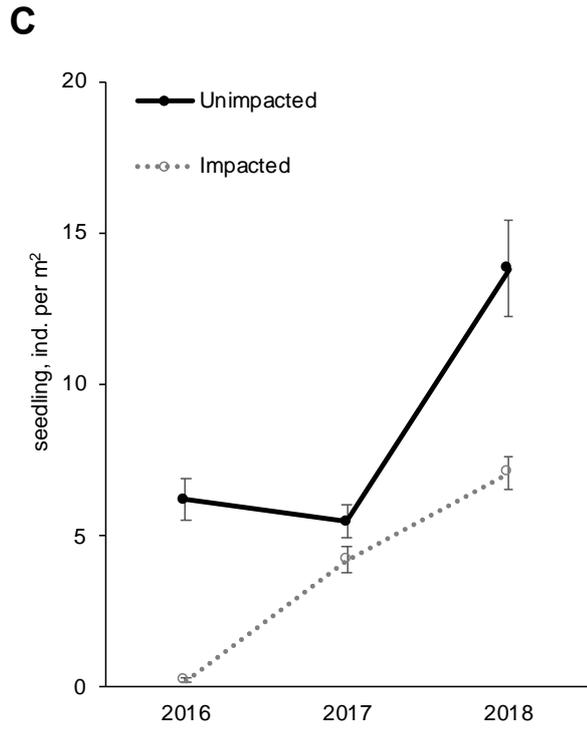
725 **Table 4.** Bulk $\delta^{13}\text{C}$ values, mean $\delta^{13}\text{C}$ values of five EAAs (%) and differences (Δ , ‰) between the two forests in 2017, 20 months after the dieback (mean \pm SD).

Group	Taxa	Forest	Bulk $\delta^{13}\text{C}$, ‰	Mean $\delta^{13}\text{C}$ of five EAAs, %	n	Δ Bulk $\delta^{13}\text{C}$, ‰	Δ Mean $\delta^{13}\text{C}$ of five EAAs, %	Permano va p value
Algal feeder	<i>Tubuca signata</i>	Unimpacted	-17.1 \pm 1.4	-21.9 \pm 1.5	3	1.7	1.4	0.90
		Impacted	-15.4 \pm 1.4	-20.5 \pm 1.8	3			
Leaf feeder	<i>Parasesarma / Episesarma</i>	Unimpacted	-21.4 \pm 1.5	-25.3 \pm 1.6	3	3.1	1.4	0.40
		Impacted	-18.3 \pm 0.2	-23.9 \pm 0.1	2			
Grazer	<i>Telescopium telescopium</i>	Unimpacted	-18.2 \pm 1.9	-24.0 \pm 1.8	3	1.5	1.8	0.80
		Impacted	-16.7 \pm 1.3	-22.2 \pm 1.1	3			
Filter feeder	<i>Crassostrea (oyster)</i>	Unimpacted	-19.3 \pm 0.4	-22.8 \pm 0.2	2	0.3	0.1	0.33
		Impacted	-19.0 \pm 0.8	-22.9 \pm 0.3	2			
Mangrove	<i>Avicennia marina</i>	Unimpacted	-26.7 \pm 2.2	-28.8 \pm 0.6	2	1.3	1.8	0.67
		Impacted	-25.4 \pm 0.1	-27.0 \pm 0.3	2			
MPB		Unimpacted	-25.4 \pm 0.8	-27.4	1	4.5	6.7	-
		Impacted	-20.9 \pm 1.2	-20.7	1			



730 **Figure 1.** The study location at Karumba in the Gulf of Carpentaria, Queensland, Australia (-17.435572S, 140.844766E; image
 provided by Google Earth). (A) Three sampling transects within the unimpacted reference site and three within the impacted
 site (shown as a white line). (B, C) Representative transects from the unimpacted (B) and impacted (C) sites. Each transect
 was approximately 200m. Samples were collected along each transect from higher to lower intertidal zones. Red indicated
 mass dieback region.

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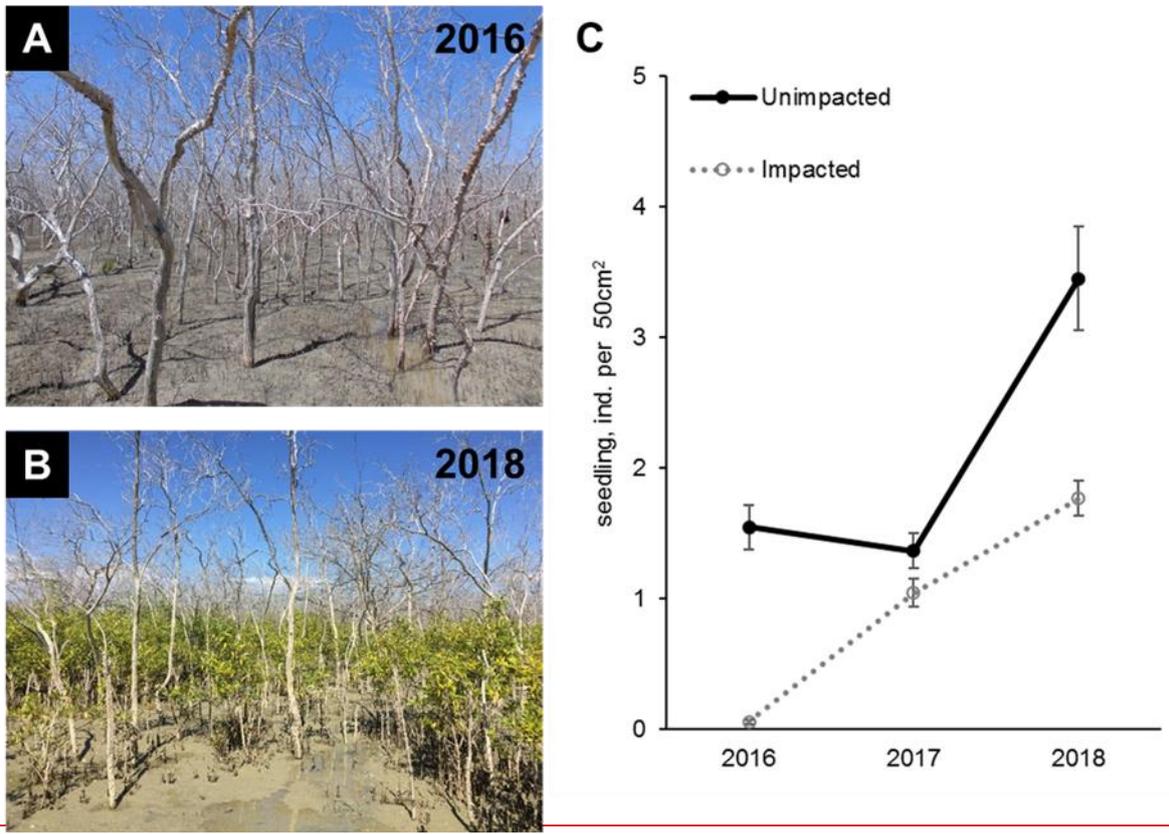


Figure 2. Recovery of mangrove vegetation at the impacted site during a two-year period from 2016 to 2018 (A and B; approx. 8 and 32 months, respectively after the dieback event). Seedling and sampling densitiespopulations of mangrove species (mostly, *A. marina*) significantly increased in the impacted site (C; Table S1).

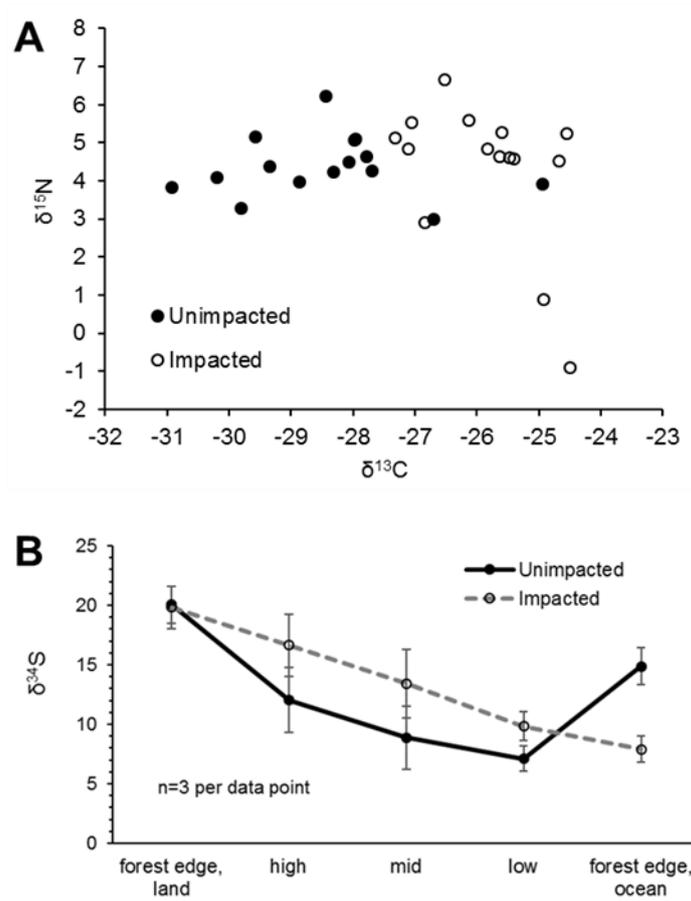
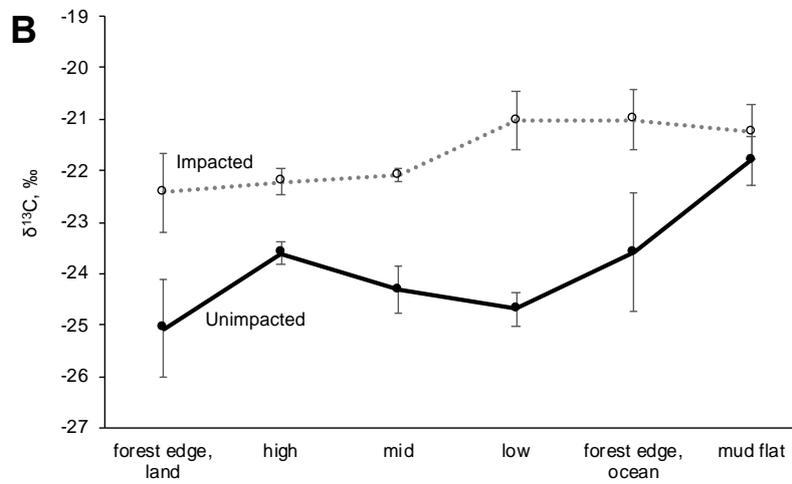
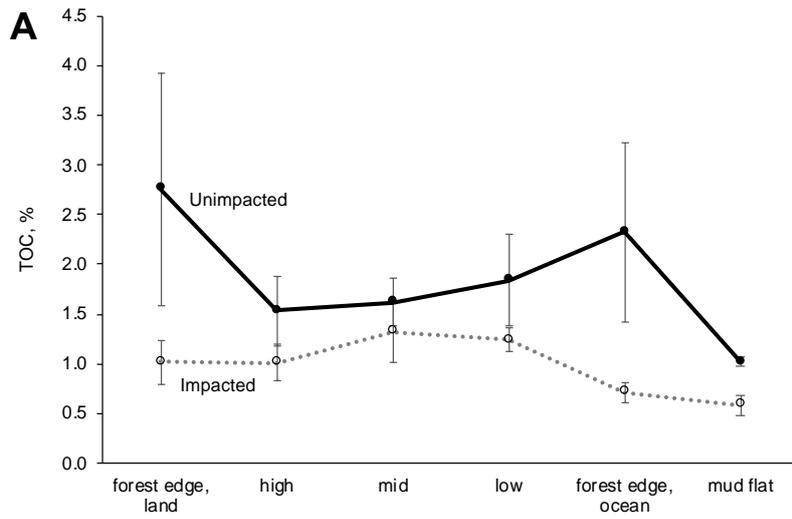


Figure 3. CNS isotopic compositions of green leaves of *A. marina* from the unimpacted and impacted sites. All samples were collected in 2018, 32 months after the dieback. (A) Leaf $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. (B) Leaf $\delta^{34}\text{S}$ values across the intertidal zones.

745 Error values are SE.



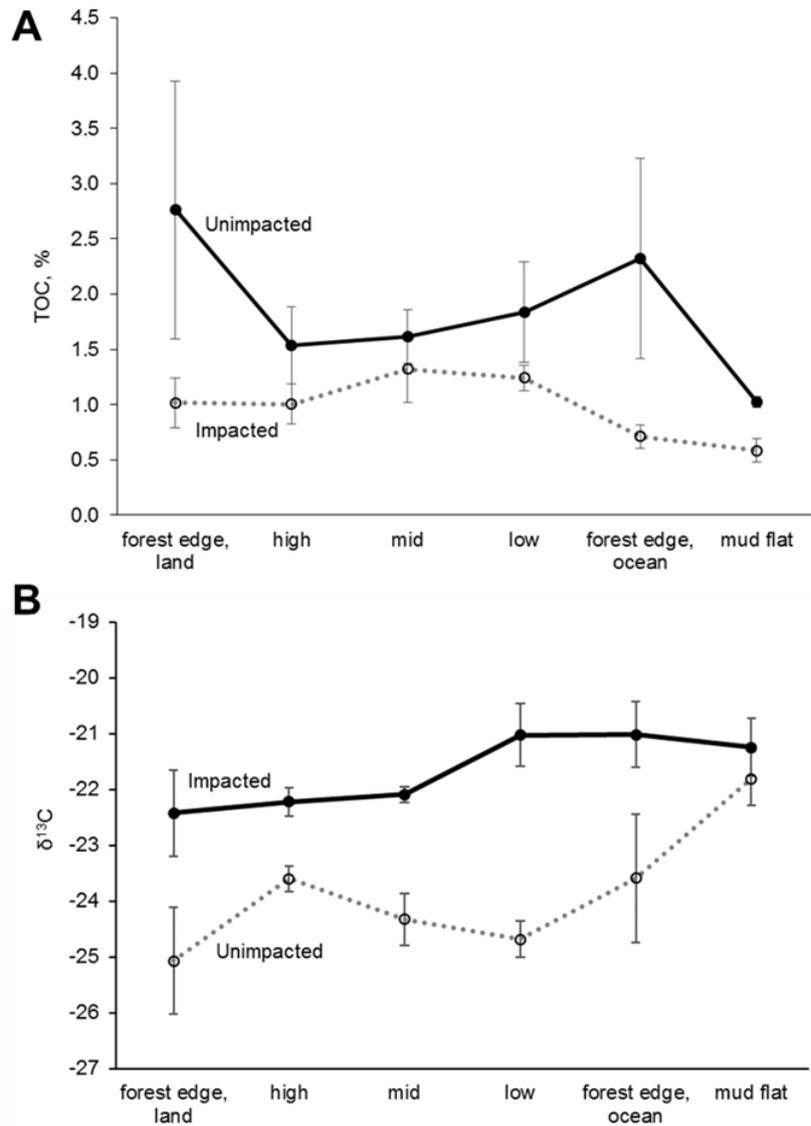


Figure 4. C elemental and isotopic compositions of surface (< 0.5 cm) sediment along the unimpacted reference transects vs impacted transects (n=3 per data point). All samples were collected in 2018, 32 months after the dieback. Error values are SE. (A) Sediment TOC, %. (B) Sediment $\delta^{13}\text{C}$ values, ‰.

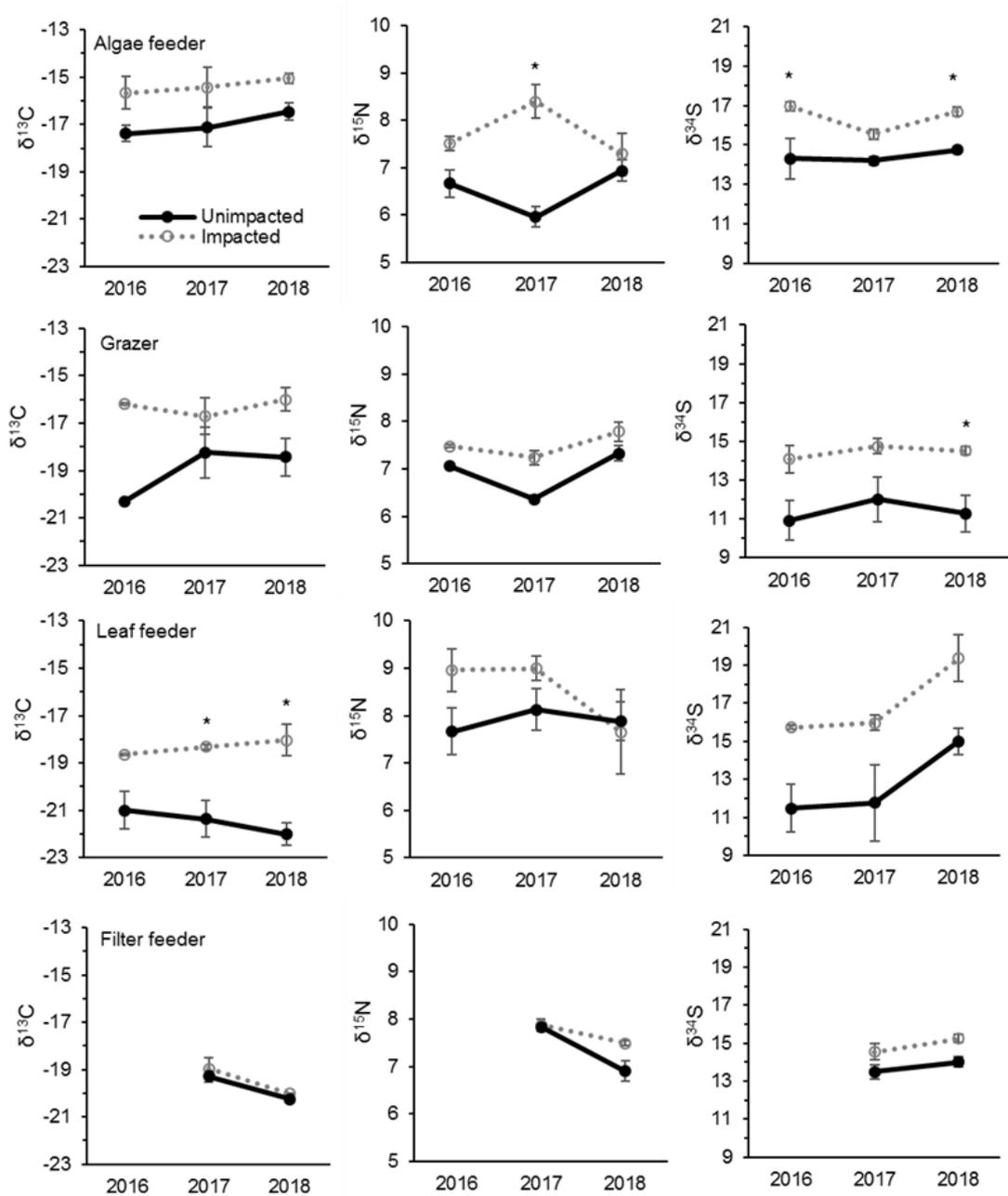
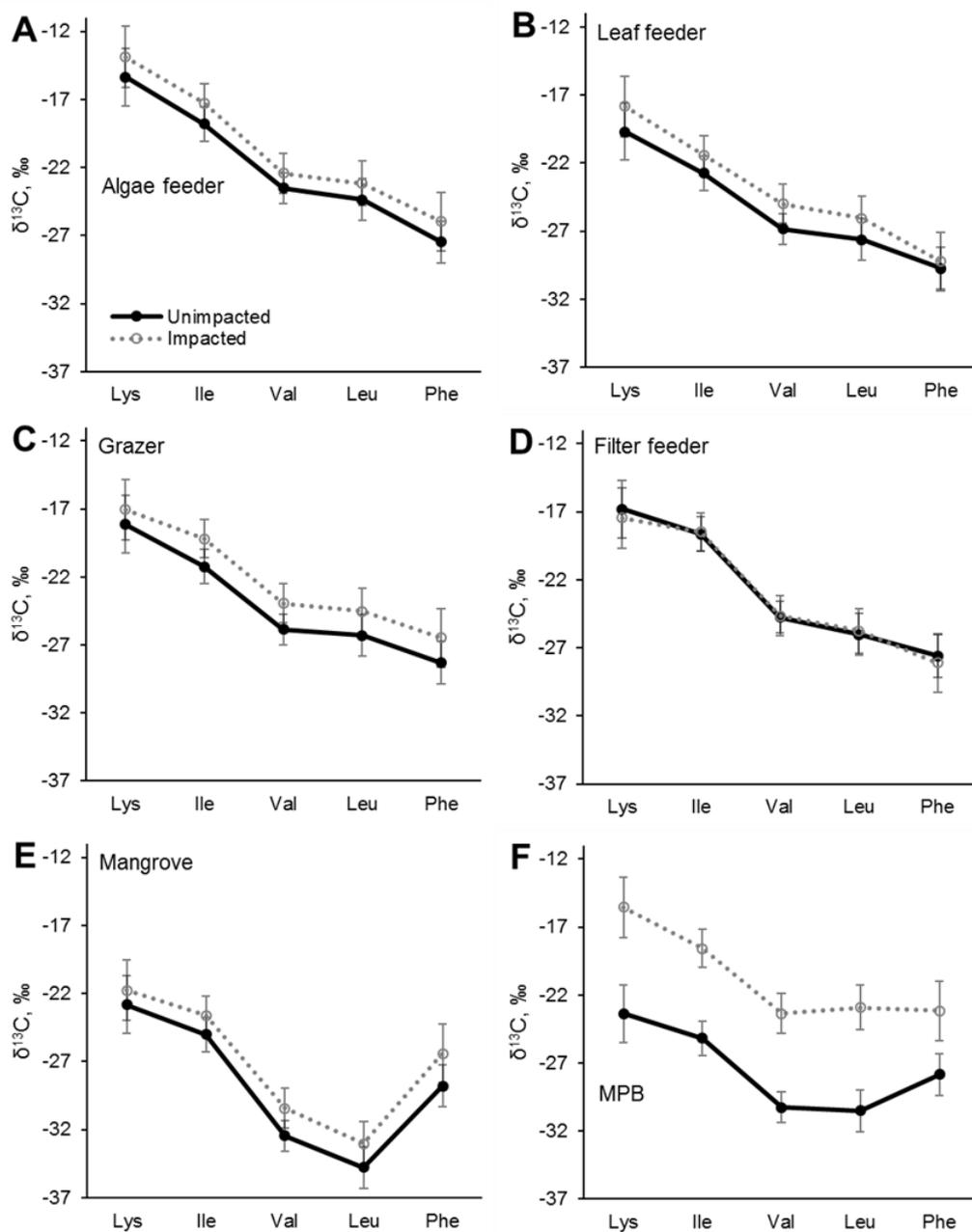


Figure 5. Changes in CNS isotopic compositions of mangrove macrofaunal groups with four different feeding modes from 2016, 2017 and 2018 (i.e. 8, 20 and 32 months after the event) between the unimpacted reference and impacted mangrove forest sites. Error bars are \pm SE (n=2 to 6 per data point). (*) indicates a significant difference in the year. Mean \pm SE values and sample sizes are also provided in Table S44.



760 **Figure 6.** C isotopic compositions in essential amino acids (EAAs) for four mangrove consumer groups and resources including mangrove leaves (*A. marina*) and MPB from the unimpacted and impacted mangrove sites during 2017 (20 months after the dieback). While there are clear offsets in individual $\delta^{13}\text{C}_{\text{EAA}}$ values between the two forests, normalized $\delta^{13}\text{C}_{\text{EAA}}$ fingerprint patterns as per Larsen (2009) shown in Fig S1 did not differ (PERMANOVA $p > 0.05$, Table 3). Error bars show \pm SD. [The data is provided in Table S5.](#)

Disturbance legacies of the forest dieback



Forest dieback due to extreme climatic events (impacted)

- $\delta^{13}\text{C}$ - weak C_3 plants signal (due to loss of mangroves)
- $\delta^{15}\text{N}$ - Moderate to high (due to degradation and lower N fixation)
- $\delta^{34}\text{S}$ - Moderate to high (lower sulfate reduction with a strong seawater sulfate signal)



Healthy mangrove ecosystem (background)

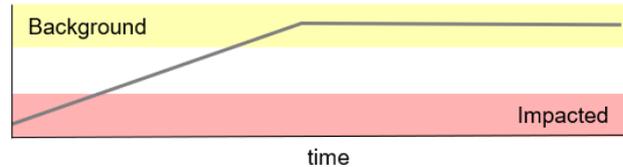
- $\delta^{13}\text{C}$ - strong C_3 plants signal
- $\delta^{15}\text{N}$ - Low to moderate (high N fixation)
- $\delta^{34}\text{S}$ - Low to moderate (high sulfate reduction)

Predicted recovery scenarios with isotopic trajectories



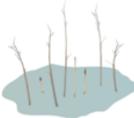
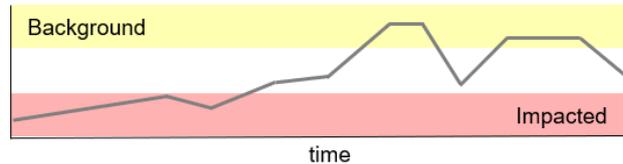
(1) Recovery with no future perturbations

- Environmental conditions allow recolonisation
- Recovery of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ to the background



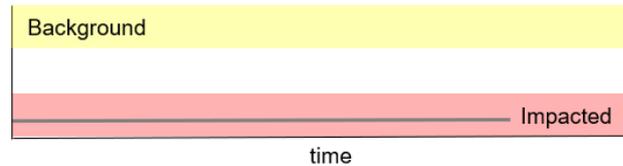
(2) Recovery with future perturbations

- Mangrove recolonisation and recovery of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$ driven by perturbations e.g. ENSO cycles



(3) Habitat becomes unsuitable for recolonisation

- Conditions not allow recolonisation e.g. due to extreme climatic events
- Transformed into intertidal mudflats
- No recovery of isotopes



(4) Incomplete recovery

- Reduced habitat size and/or recolonised by other plants such as saltmarshes
- Incomplete recovery of $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ and $\delta^{34}\text{S}$

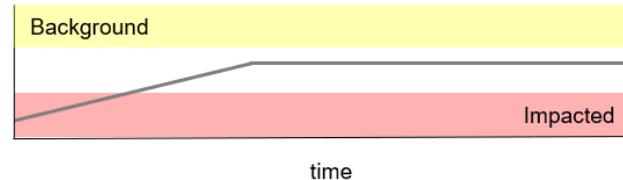


Figure 7. A conceptual diagram showing the ecological and biogeochemical legacy of the mangrove forest dieback in the Gulf of Carpentaria and four predicted recovery scenarios of the mangrove ecosystem with isotopic trajectories (δ represents the isotope values denote for of animals, plants and sediment).