

Biogeosciences Discuss., author comment AC1
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Reply on RC1

Alice E. Webb et al.

Author comment on "Quantifying functional consequences of habitat degradation on a Caribbean coral reef" by Alice E. Webb et al., Biogeosciences Discuss.,
<https://doi.org/10.5194/bg-2021-64-AC1>, 2021

Comments from reviewers:

Referee #1 (Comments to the Author):

This is a very interesting paper that addresses biogeochemistry among five different substrate types in the Caribbean using closed in situ tent incubations. There is a wealth of data here that would be great to see published in Biogeosciences. However, I have several key concerns in the methods that should be addressed before this work is considered, mostly relating to the low units of replication, and consequent analyses, narrative and overall extrapolation of reef functioning thereafter.

It seems that the inverse model outputs employed distract from more targeted analyses and presentation of, what could be, quite compelling results on biogeochemical fluxes in the tents among substrate types. This modelling approach may have been adopted owing to the low sample size in the study; $n=3$ daytime and $n=2$ nighttime per substrate type. If so, the authors should express this clearly. While I cannot speak for the models themselves, as this is far from my expertise, I am concerned that the models were established based on just 2-3 replicates per treatment. In fact, calculations of 95% CIs (e.g. Fig 6) were also inferred from model outputs owing to low sample size. It is likely that the low level of replication within each substrate type ($n=2-3$) skews the overall result towards "no significant differences between any communities" (Ln 243), when this is not actually the case. This appears to be a key take-home message (e.g. abstract Ln 28; "no significant difference between processes on any assemblage") that may not be true, but there was not enough power to show otherwise. This forms a narrative and conclusion that may dangerously misrepresent the data, which poses significant risk when extrapolating findings to ecosystem functioning and functional redundancy.

We thank the reviewer for this relevant remark. The modelling approach used in this study to estimate biogeochemical processes was first and foremost to relate the measured change in concentrations of variables (A_T , DIC, etc.) to the responsible metabolic processes. The reviewer is correct in pointing out that the limited number of replicates may be insufficient to permit the use of a PERMNOVA to analyse our data. Although we group the incubations in five different categories based on the dominant group, we do acknowledge that these

communities do harbour differences in terms of composition and therefore refrain from performing analysis using the community composition as a categorical factor.

In line with the reviewer's key concerns:

- **we removed the claim of functional homogenisation**
- **altered the title to put a bit of emphasise on the methodology aspect of the paper, it now reads:**

'Quantifying functional consequences of habitat degradation on a Caribbean coral reef'

- **We removed the PERMANOVA analysis (see below) and replaced it by PCA was conducted on a centred multivariate data set consisting of the four main biogeochemical processes (i.e., NCC, NCP, nitrification and denitrification).**
 - **We rephrased some parts of the introduction and discussion and included a more cautious interpretation of the results.**
 - **Although for visual ease, we do group the incubations in 5 different categories (with various colours), we refrained from performing analysis using composition as a categorical factor, rather treating each incubation as an individual.**
-
- **The reviewer is correct in pointing out that calculating 95% CI on only 2 replicates is not relevant. Instead, we plot figure 6 as a scatter plot depicting all estimated parameters with their mean.**

The inverse modelling seems to overlook specific results among treatments. Table S2 presents model output data, but this does not compare treatments/factors (day vs. night, substrate types). Such comparative analyses are found in Table S3, which is the PERMANOVA result that shows no significant differences, except for day vs. night. It is unclear what data (factors or response variables) were even used for the PERMANOVA, which I feel may not be the correct approach to analysing these data.

The PERMANOVA analysis has been removed.

I would argue that if more targeted analyses (e.g. linear models, ANOVA) were conducted, say for NCP or NCC between day-night and substrate types, more informative results would emerge. For example, in Fig 6, NCP is much lower at night in coral, BES and BCM than in sand and TMA. Conversely, NCC is greater in the day for coral, BES and BCM than in sand, and lowest at night for BES and BCM. I am just eyeballing data here, but this does not look like "no significant differences between any communities" (Ln 243). I am not sold by the PERMANOVA, as it seems there should be some detectable differences between substrate types, and that greater detail and resolution could uncover this.

The PERMANOVA was removed. Due to limited numbers of incubations, we also didn't perform an ANOVA using substrates as a categorical factor.

Instead, we performed a PCA (now figure 7) based on the four main biogeochemical processes estimated for each incubation. We added text to the methods I. 243:

Principal component analysis (PCA) was used to identify grouping among the 23 tent incubations (day n = 13, night: n = 10) in relation to their biogeochemical signature (i.e., NCC, NCP, nitrification and denitrification). PCA was conducted on a centred multivariate data set consisting of the four main biogeochemical processes (i.e., NCC, NCP, nitrification and denitrification).

And in the results I. 309:

The PCA based on the four main biogeochemical processes revealed two main different groups between night incubations and day incubations (Figure 7A). Sand incubations were the exception as night and day incubations grouped relatively close to each other. The first two principal component axes (PC1 and PC2) explained 88.68% of the total variability within the data. PC1 described a gradient in NCP and NCC from high (negative PCA scores) to low (positive PCA scores) and an opposite pattern for nitrification and denitrification. PC2 further explains the variability in NCC and nitrification, and to a lesser extent NCP and denitrification. One of the communities dominated by bioeroding sponges (rep.1) is separate from other communities both during the day and during the night due to high rates of nitrification and denitrification compared to other communities.

Also, it seems that the dominant substrate type was used to categorise factors, but this may impact (and limit) the results due to low sample size. One alternative to this issue could be to analyse all tents using continuous data for substrate type. E.g. could data be analysed at the level of "percent cover of sand", "percent cover of coral", "percent cover of cyanobacteria", and so on... rather than treating them as fixed categorical factors? This would increase sample size, and possibly tease out interesting results e.g. thresholds of cover for positive or negative results regarding ecosystem processes and functions. Otherwise, more data / replicates may be needed. I fear that in using the current approach, conclusions are made on ecosystem functioning and redundancy that extend beyond the scope of the data.

Although we considered this proposition very seriously, and tried out potential analysis accordingly, the present experiment protocol was not built to answer this question and therefore analysing data at the level of percentage of sand/bcm/coral... is problematic as the percentage of cover of different groups in the incubations is not continuous. For instance, coral is only present in coral and bioeroding sponge dominated communities, and cyanobacteria are only present in BCM dominated tents. Getting thresholds of cover for positive or negative results regarding ecosystem processes is challenging due to the large gaps between percentage cover of various groups (e.g., for sand, the highest

cover is 100 % followed by 24 %). Hence, we refrained from such an analysis.

Abstract

Abstract Ln 23-33: I suggest changing to past tense here, e.g. "Estimated processes were low" and "No real gain in primary habitat was recorded".... And so on.

We have made changes accordingly.

Ln 28: Suggest removing reference to the analysis here "A multivariate pairwise analysis revealed that there is no significant difference..." to be more succinct, e.g. "We found no significant difference..."

We removed this sentence because we removed the PERMANOVA. Instead, we write at l. 32:

Results suggest similar directions and magnitudes of key biogeochemical processes of distinct communities on this shallow Curaçao reef.

Introduction

Ln 36: "habitant" should be "habitat"

We have made changes accordingly.

The authors should consider other relevant literature on coral reef ecosystem functioning and functional redundancy, e.g. - Range of work by Bellwood,

e.g.: o Bellwood, D. R., Hoey, A. S., & Choat, J. H. (2003). Limited functional redundancy in high diversity systems: resilience and ecosystem function on coral reefs. *Ecology letters*, 6(4), 281-285

Bellwood, D. R., Streit, R. P., Brandl, S. J., & Tebbett, S. B. (2019). The meaning of the term 'function' in ecology: a coral reef perspective. *Functional Ecology*, 33(6), 948-961 - Wolfe, K., Anthony, K., Babcock, R.

C., Bay, L., Bourne, D. G., Burrows, D., ... & Mumby, P. J. (2020). Priority species to support the functional integrity of coral reefs. *Oceanography and Marine Biology*.

We thank the reviewer for these relevant publications, we added them to the introduction I. 63, 74 and 61 respectively.

Ln 70: Additional work could be considered including in situ and lab experiments,

e.g. - Albright, R., Caldeira, L., Hosfelt, J., Kwiatkowski, L., Maclaren, J. K., Mason, B. M., ... & Caldeira, K. (2016). Reversal of ocean acidification enhances net coral reef calcification. *Nature*, 531(7594), 362-365

- Albright, R., Takeshita, Y., Koweek, D. A., Ninokawa, A., Wolfe, K., Rivlin, T., ... & Caldeira, K. (2018). Carbon dioxide addition to coral reef waters suppresses net community calcification. *Nature*, 555(7697), 516-519

- Dove, S. G., Kline, D. I., Pantos, O., Angly, F. E., Tyson, G. W., & Hoegh-Guldberg, O. (2013). Future reef decalcification under a business-as-usual CO₂ emission scenario. *Proceedings of the National Academy of Sciences*, 110(38), 15342-15347

- Dove, S. G., Brown, K. T., Van Den Heuvel, A., Chai, A., & Hoegh-Guldberg, O. (2020). Ocean warming and acidification uncouple calcification from calcifier biomass which accelerates coral reef decline. *Communications Earth & Environment*, 1(1), 1-9

- Brown, K. T., Bender-Champ, D., Achlatis, M., van Der Zande, R. M., Kubicek, A., Martin, S. B., ... & Hoegh-Guldberg, O. (2021). Habitat-specific biogenic production and erosion influences net framework and sediment coral reef carbonate budgets. *Limnology and Oceanography*, 66(2), 349-365

We thank the reviewer for these publications, we added these papers to the introduction I. 77,77, and 84,84 respectively.

Methods

Ln 92, 93, 95, 144, 150, 184, etc... Suggest making methods section past tense e.g. change "is" to "was", "are" to "were", etc

We have made changes accordingly

Ln 102: More information should be provided on the number of replicate tents used per substrate type;

- There were five substrate treatments, each with three replicates (?) (Fig 2).

Yes, we have rephrased the sentence I. 118. It now read:

Incubated communities included five different types of substrate dominated either by turf and macroalgae (n=3), sand (n=3), bioeroding sponges (n=3), benthic cyanobacteria mats or coral (Fig. 2), equalling a total of 15 studied communities. Each community was incubated during the day (n=15) and due to practical reasons, only 2 of each type were incubated during the night (n=10) (i.e., for each type of community, three daytime and 2 night-time incubations were carried out).

Were all of these deployed at the same time or was a single custom tent reused for all?

We used two custom tents which we interchanged so that they both spent some time out of the water to avoid fouling. Only one incubation was performed at a time due to limited Oxygen, CTD and pump equipment.

How long were tents left over the substrate before beginning the experiments / incubations?

Prior to each incubation, the tent was place with flaps open over the substrate and lefts for a minimum of 3 hours before the incubation was started to permit the community to get to acclimatise and sand to settle. We have added a paragraph with this information (also for the question above) I.124:

The incubations were carried out one at a time, over the study period and lasted four hours each. Prior to each incubation, the tent was place with flaps open over the substrate and lefts for a minimum of 3 hours before the incubation was started. When day incubations were terminated, the tent was left in place with flaps open until the night incubation was carried out on the same substrate. All daytime incubations were started at 10:00 and all night-time incubations were started at 18:30.

Was the substrate left to stabilize in cases where substrate was moved into the tent to artificially construct the benthic community?

In these cases, the community was left to stabilise two or three days before starting incubations. This information is now added I.133.

Daytime incubations were done in triplicates (Ln 102) and night incubations in duplicates (Ln 102), but is this n=3 tents per substrate incubated 3 (day; n=9) and 2 (night; n=6) times or just one tent done n=3 (day) and n=2 (night) times?

o If the former, was tent number incorporated as a factor to account for pseudo-replication of tent/substrate type? And were there differences detected in seawater parameters across incubations (i.e. repeated measures?)

o If the latter, units of replication per treatment and timepoint are quite low.

To make this clearer, we have added the paragraph to section 2.2 (lines 118 and on):

Incubated communities included five different types of substrate dominated either by turf and macroalgae (n=3), sand (n=3), bioeroding sponges (n=3), benthic cyanobacteria mats or coral (Fig. 2), equalling a total of 15 studied communities. Each community was incubated during the day (n=15) and due to practical reasons, only 2 of each type were incubated during the night (n=10) (i.e., for each type of community, three daytime and 2 night-time incubations were carried out).

Ln 103: Incubations went for 4 hrs each, but did they all start around the same time day and night? It seems in Fig 5 that O2 does not follow the trend line selected, but instead has large fluxes across the 4 hrs. Does this reflect differences in incubation time, perhaps started later in the morning or afternoon than others..? Perhaps add a sentence like "all daytime incubations were started at X am, and all nighttime incubations were started at X pm".

Yes, day incubation all started at 10:00 and night incubation at 18:30. We have added this to I. 127.

All daytime incubations were started at 10:00 and all night-time incubations were started at 18:30.

Ln 116: Species name must be italicized

We italicised the species *Oscillatoria bonnemaisonii* now l. 140.

Ln 134: Were filters changed between samples and incubation time points? If so, how? If not, could sediment and particles trapped in the filters from T0 have impacted samples at T2 and T4?

Filters were changed regularly but not during incubations. When filters were changed, they were inspected and did not show over saturation of sediment. We visually inspected every single alkalinity samples before processing them on the optical titrator. The sand found at this site consists mostly of carbonate. Even with small grains of sediment present in the samples, they will dissolve after addition of acid and result in pronounced peaks in alkalinity. Since this did not occur, we are positive there was no sediment in the samples. We have added the sentence below to explain these filters were changes regularly l. 159.

The tube end located inside the tent was equipped with a Whatman ® filter (G/F 0.47 µm) which was replaced daily.

Ln 203-207: Be clear if benthic cover (dominance) was used as a fixed categorical factor in analyses. If it was, how may the difference in cover for algal dominance (72-83%) or cyanobacteria dominance (83-91%) have influenced results from these respective tents? I believe that a 10% difference in algal or cyanobacteria cover could be quite influential. This seems like an important consideration given that there were possibly just n=3 replicates per substrate type. (See major comment above).

As the reviewer recommend, we have changed our statistical analysis. See answer to major comment above.

Ln 205: Was PERMANOVA conducted on raw or model data? This is important to state. I am not sure how robust this analysis is to such low sample size n=2 within raw data, but also unsure whether such analyses should be conducted on modelled data. Further, what biochemical processes (variables) were analysed using PERMANOVA? Table S3 shows just one set of output data, but how does this translate to NCC, NCP, etc? What exactly was tested here?

We have removed the PERMANOVA analysis, see above.

Results

The results would benefit from a few subheadings to form structure. E.g. #1 general temperature, salinity, light in the tents – baseline conditions / tent effects, #2 incubations with differences in AT, CT, pH, N, etc. among tents and substrate, and #3 NEC and NCP among tents and substrates.

We have added 5 subheadings accordingly.

3.1 Ambient conditions

3.2 Water exchange quantification

3.3 Model output

3.4 Estimated biogeochemical processes

3.5 Incubation comparison

Ln 215-218: How did these leakages impact incubations? Given the exchange rates were greater in these two tents, is it possible that their leaking confounded the results from these tents? How was this accounted for?

We checked for correlation between processes and the amount of leaking using the Kendall rank correlation test. No significant correlation was found. Text was added in the methods I. 240:

To evaluate if water exchange rate had an impact on estimated processes, the non-parametric Kendall rank correlation test was performed. All inferred biogeochemical process rates (mineralisation, primary production, NCP, NCC, nitrification and denitrification) were tested against incubation water exchange rates.

and the results I.303:

The Kendall rank correlation test did not reveal significant correlation between water exchange rates and rates of mineralisation ($p=0.79$, $\tau=0.04$), primary production ($p=0.47$, $\tau=0.12$), NCP ($p=0.75$, $\tau=0.05$), NCC ($p=0.17$, $\tau=0.21$). Nitrification ($p=0.81$, $\tau=0.04$), and denitrification ($p=0.27$, $\tau=0.18$). The Kendall correlation coefficient τ is closer to zero than 1 in all cases, implying there is no significant association between the two tested variables.

Ln 227, 228, 231, etc: Again, I would stick to past tense in results section, e.g. "NCP showed a clear diurnal pattern"

We changed the tense accordingly.

Figure 5: This figure looks like a copy-paste model output. Minimum, panels should be better labelled. However, a more informative figure could be produced that summarises the model outputs for the five substrates, day and night.

Figure 5 now presents model outputs for the five substrates, day and night. The old Figure 5 was made to give an illustrative view on the model output and what parameters were involved. We therefore placed it in the supplements (now Figure S1).

Figure 6: Panels should be labelled with A, B, C and D. Also, it now seems that Table 1 is redundant given this information is provided here in Figure 6. It is much easier to view as a figure, so I suggest deleting Table 1

Figure 6 has been labelled accordingly and table 1 has been deleted.

On this note, it would be nice to see other seawater chemistry data (AT, CT, pH, etc) presented like this in a separate figure or table. I feel the results are short and overlook baseline measurements of seawater chemistry among tents and substrate types. How did AT, CT, pH vary among substrates and across incubations?

All measurements are now depicted in Figure 5 but the data was centred to illustrate the different changes between incubations. A new table has been created showing all measured variables throughout incubation, day and night. It can be found in the supplements, Table S2. Text has been added I.254:

Average ambient AT, DIC, pH, NH₄ and NO₃ was 2386.8 ± 13.9, 2125.5 ± 20.0, 7.9 ± 0.003, 0.31 ± 0.15 and 0.32 ± 0.14 μmol kg⁻¹ respectively. Measured data for each incubation, inside and outside the tent for all three time-points, as well as the differences between T₀ and T₄ are presented in Fig. S2.

Ln 242: "per" should be capitalised in PERMANOVA

We removed the PERMANOVA analysis.

Discussion

Ln 253: This is a nice contrast, but make it clear by stating the ranges of NCC you found, as well as that commonly found in the literature.

This is the part of the discussion summarizing findings. More detailed ranges are stated l. 364 and onwards.

Ln 254: What does "no real gain in primary habitat" mean? Do you mean reef accretion / coral growth? If so, use this very carefully, as NCC and accretion are not always coupled. Low (or high) rates of NCC do not always correspond to low (or high) accretion rates. Did you measure primary habitat gain somehow, or is this assumed from NCC rates?

We assume gain in primary habitat from NCC rates. Here we wanted to make clear what these processes translate to in terms of function, but the reviewer makes a relevant remark, we have rephrased this sentence to moderate this statement. (l. 330)

Very low or negative NCC rates were recorded on all substrates indicative of reduced net accretion potential.

Ln 255: As above, saying "accumulation of biomass through photosynthesis is low" may not be true.

This was an attempt to translate biogeochemical processes into functions to

make it clear why we investigate these. We have now changed the sentence I. 331 to:

'Net production was also low, likely indicating limited accumulation of biomass, while heterotrophic...'

Also, use past tense throughout: "was" not "is", "were" not "are"

We changed the tense accordingly.

Ln 260: I am not convinced by this statement. Fig 6 shows differences among substrates, which more explicit analyses may reveal. Functional homogenisation is quite a loaded term to use from n=2-3 replicates.

We have revisited our analysis as advised by reviewer and removed the overall mentioning of functional homogenisation.

Ln 267, 280, 298, etc: Consider a new paper that addresses calcification and dissolution on dead and live coral surfaces: - Romanó de Orte, M., Koweek, D. A., Cyronak, T., Takeshita, Y., Griffin, A., Wolfe, K., ... & Caldeira, K. (2021). Unexpected role of communities colonizing dead coral substrate in the calcification of coral reefs. *Limnology and Oceanography*

We thank the reviewer for this very relevant paper (with comparable results). We added it to our discussion I. 447.

Ln 269: In reference to my comment above, how was "accretion rate" calculated?

This sentence was removed.

Ln 274: "an" should be "and"

This was changed accordingly.

Ln 275: "Koweet" should be "Koweek"

This was changed accordingly.

Ln 290-293: This text is useful but has no clear point as currently expressed. I also see its relevance at Ln 365.

We rephrased this sentence 1.472 to improve clarity and placed it at the end of the discussion as suggested by the reviewer. It now reads:

Additionally, average ambient pH at the current study site was 7.9 which is lower than average 'summer' pH, usually between 8.1 and 8.2 (den Haan et al. 2016). This may suggest that depressed calcification rates in the Piscadera Bay are indeed linked to seasonality. However, further research and additional incubations are needed to better understand the seasonal component of reef functions.

Ln 341: As above, this key message may not be correct given the low sample size and unclear PERMANOVA analysis. The narrative and analyses must be readdressed.

see above

Ln 345: This level of 34-36% coral cover is very high for the Caribbean. Why was this done? Were corals intentionally moved into tents to create this high coral cover? If so, were they left to stabilize for several days after relocation? Were any metrics of coral condition measured before (and/or after) incubations? Is it possible that the corals were stressed and under-performing?

Corals were not moved inside tents, we did add some substrate with bioeroding sponges or turf. But never corals. No metric of coral condition was performed, we inspected them visually, they looked healthy before and after incubations. No release of mucus was observed and coloration still present. Our results suggest that the corals in this site are indeed impacted by the organic matter overload in this area.