

Referee#2

Nice study overall, and a valuable contribution.
Some edits suggested for clarification.

We would like to thank referee #2 for her/his thoughtful comments, and are grateful for the effort placed into reviewing in great detail the content and wording of our manuscript. We are pleased that she/he found out study nice and valuable and will address his/her comments and edits in detail below.

A concern, which can be addressed, is over use of the term "preference" indicating observation of higher density of colonization on some substrates, being over interpreted to prove preferences for specific cations or anions.

There are many variable factors in a natural environment. First looking at distribution in a quantified manner allows hypotheses for testing to illustrate actual "preference"

In hindsight, we agree that this term definition involves already a level of interpretation that is not adequate or useful to describe the results, we will therefore reserve that term for the discussion part.

bg-2016-254 Edit Suggestions and comments

Line 21: define "dominant" as compared to what? Phototrophs? OTUs? Biomass

Line 27: substitute "suggesting" for "calling for"

Line 44: MacIntyre et al, describe a two phase boring and subsequent new crystal formation within boreholes (within grains and connecting to adjacent) rather than external cementation of grains.

Line 48: m-2 d-1 and are of clear

Line 73: end sentence at "including genetic markers." Delete (a task yet to be undertaken with any breadth) or re-word at the beginning and add "To date" these genera were all assigned....

Line 77: suggest deleting "alternative" and edit to "complimentary and more comprehensive descriptions" of endolithic....

Line 79: delete "merely" and "morphological studies alone"

Line 81: delete "brought to our attention" substitute "have revealed"

Line 82: delete "not just composed of" substitute "in addition to"

Line 21: Here we meant "dominant" compared to

Line 27: We will do so in the revised version of the manuscript.

Line 44: "*the cementation of loosely bound carbonate grains in coastal stromatolites*" We would like to thank referee #2 for pointing out these details, we agree that the use of the term "cementation" implying the trapping of the grains by an external matrix does not describe accurately the process at play. We will rephrase this sentence as follow in the revised manuscript: "*the formation of lithified laminae of welded carbonate grains in coastal stromatolites*"

Line 48: ok

Line 73: We will add “To date”

Line 77: Good suggestion. We will replace “alternative” by “more comprehensive”

Line 79-82 We agree to these edits.

Comment: this section on associations and succession patterns is well placed

Line 90: Suggest re-wording “However similar studies using the power of high throughput sequencing techniques are not yet available for the globally....”

Line 100: delete “could show” substitute we “found”

Line 101: delete “fastest” substitute “most rapidly”

Line 106: delete “arguments” suggest rewording “Similar substrate preferences have also been observed for phosphates”

Comment: lines 110 to 115, intriguing and compelling questions.

Line 112: Suggest re-wording. Perhaps, “We therefore developed the line of inquiry to ascertain if evolutionary specialization has resulted in a highly adapted....”

Line 114: Suggest deleting sentence beginning with “Surprisingly, this aspect....”

The previous line is stronger without Line 114.

Comment: Lines 117 to 123, nicely and succinctly phrased.

We are pleased that referee #2 found some sections well placed, well written or even compelling enough to be mentioned.

Line 90: We will reword this sentence as follow: “*“However, no high throughput sequencing studies are available on the globally significant intertidal endolithic communities.”.*”

Line 100-101-106: We agree to these edits.

Line 112-114: We would like to keep these two sentences as they are because we think that it is important to state very directly the originality of the present study in the framework of the existing literature especially for a non-specialist audience.

Question, line 135: Was the geologic hammer sterilized before sampling? - See that sterilization steps were taken in the lab in line 157.

Line 137 and paragraph: seems awkward and a little confusing. What were replicates?

Perhaps "At each sample location three replicate aliquots of rock chips were collection in sterile 50 mL falcon tubes."

Presumably Air drying was done in the lab? And preservation in alcohol in the field?

Text should clarify this here. Also please specify how long in transit and transit conditions (light/ dark?/ .

Where were the seawater aliquots collected relative to the sample locations? (add to map in Fig 1.) and specify how handled/ stored and time to analysis.

Line 149: Could delete the word "retrieve"

Line 158: A chip was further "ground" not "grounded"

Line 161: was modified "by homogenizing bead tubes" delete "as follow" and delete "were applied"

Line 176: "were performed"

Line 182: "barcode" removal

Line 184: processed

Line 188: "further report specific abundances for each sample"

Line 135: To ensure or at least minimize contamination in the field, the hammer was thoroughly washed with surrounding sea water at each sampling point. The surfaces of all samples were then thoroughly brushed with sterile implements in the laboratory to eliminate all surface epiliths (and contaminants).

Line 137: Each sample was broken down to three pieces that were stored differently depending on downstream planned analyses. For each sampling location the samples used for mineralogical and biological analyses constitute biological replicates, we will clarify this point in the revised manuscript.

Air drying and alcohol preservation were both done in the field. Samples were transported in the dark at room temperature for 5 days before

Sea water samples were collected in sampling site K (west coast). Seawater was collected in a sterile polypropylene bottle, filtered on site on 0.22 μm sterile filter and stored at 4°C in the dark. After 5 days of transit at room temperature in the dark it was stored back at 4°C in the dark for an additional week before being processed.

Line 149-158-161-176 We will perform these changes

Line 182 There is one barcode per sample.

Line 184 -188 Of course we will correct this two, thank you.

Line 189: Suggest “Because this study focused on the most abundant OTUs and how they vary, rather than the rare biosphere, we filtered....to remove the **few?** rare OTUs”

Question how many rare OTUs were present?

Line 207: Suggest, “For comparison, raw sequences....”

Line 212: delete “they were” substitute “and also” delete “as well”

Line 222: “ran”

Line 254-256: worded a little awkwardly,

Line 290: communities “in this study” are much more complex than the majority of literature “to date”

Line 294: proven that “some axenic” cyanobacteria delete “alone”

Line 296: other metabolic activities (of other co-occurring microorganisms) particularly those that result in pH changes....

Line 189 We removed a lot of rare OTUs, as mentioned in the text we analyzed only 11% of the total number of OTUs that were originally generated. However, the 89% OTUs that we removed accounted for less than 10% of the total sequences altogether (this information is line 193) .

Line 207-212-222 We will perform these changes

Line 254-256 We will reword this section as follow “*Isla de Mona was never continuously inhabited. The island was mostly used as a guard post for the Mona Passage throughout the 20th century, and declared a Nature Preserve in 1993 (National Parks Register, USA) .*”

Line 294-296 We will perform these changes

Comment Lines 307-320 Nice discussion

Line 349: delete "does" ie., "one that not only represents" an initial set of pioneers...

Line 356: delete "always" substitute (has "so often" been described...)

And delete "who can" substitute "accounting" ie., "community accounting for"

Line 360. "were" instead of "was"

Question: are attempts in progress to isolate *Plectonema terebrans*? And are isolates available from other sources?

Minor editing lines 385 to 403

Line 404 delete "It becomes clear that substrate preferences" suggest substitute "Results suggest substrate preferences are found..."

Some rewording of section line 410 to 414, probably split into two sentences

Line 417 suggest "although the paucity of samples restricted our statistical power, we were still able to identify..."

Line 422: suggest delete "promiscuous" which is vague substitute "widespread across different substrate types"

Line 307-320 Thanks

Line 349 We will delete "does"

Line 356 As far as we know *P. terebrans* has always been described as an important player of endolithic communities so we will keep the wording.

We will rephrase the next sentence using "accounting for"

Despite our efforts we could never isolate *Plectonema terebrans*. We don't know of any other groups trying to do so at the moment, there are no isolate available.

Line 404-422 We will perform these changes

Comment: At the beginning of the manuscript “substrate preference” refers to clear numerical/ statistical occurrence (of particular endolithic cyanobacterial species relative to other species) in different mineral/ rock types. However at this point in the manuscript the term is “preference” is seeming to take on a more determinative meaning that is not yet demonstrated. Preference can be a tricky term to use. implying a more “decision based”

A particular species may seem to show a preference for a Mg or Ca cation containing mineral substrate based on occurrence/ density, but that does not necessarily imply a “preference” for Mg or Ca cations. That would need to be tested independently, as would the “preference” for anions.

The authors seem to understand this, but still sometimes fall into an overly interpretive phrasing implying metabolic/behavior from a distribution.

Certainly the data suggest some trends worth rigorously testing, (as has been done for *M. testarum* BC008) and it is critical to begin the determination of substrate preferences, by detailed investigations of naturally occurring distributions as the authors have done.

Line 447: in light of previous discussion would suggest changing “preference” to

“NR_OTU193) did show a **higher rate of occurrence** in calcium carbonates as compared to magnesium carbonate.

A careful re-reading and edits with particular emphasis on the implications and possible over interpretation of the term “preference” would be very beneficial.

Line 452: delete “we must assume” again data “suggests” but does not “prove”

Comment: Authors conclude that more factors may be involved in substrate preference that cation preference alone. A bit more discussion of what those other factors may be would be helpful.

Perhaps also in the introduction a short synopsis on thought / previous research as to “why” boring behavior is prevalent in some groups would be informative (ie., is behavior thought to provide protection from wave energy/ nutrients/cations/ light modification)?

We agree with referee #2 that we overused the word “preference” and we will carefully review the whole manuscript with that in mind, replacing the term by more accurate descriptive terms such as “rate of occurrence” or “relative representation” each time that we can.

Following referee#2 advice, here is sentence we would like to add to the discussion to suggest alternative boring mechanisms:

“These contrasted findings highlight that there must be factors other than the cationic part of the mineral determining the excavation ability of a particular strain and that the boring mechanism proposed for *M. testarum* strain BC008 might be only incompletely described. *Other mechanisms have been suggested to*

explain boring mechanisms which have been invalidated for the model organism M. testarum strain but may prove themselves valuable for other taxa. The dissolution of carbonate mineral by acid excretion was proposed by (Haigler, 1969) and (Golubic et al., 1984). This mechanism could involve spatial or temporal separation of photosynthesis vs. respiration by cyanobacteria or acid production as a byproduct of other heterotrophic bacteria activity (Garcia-Pichel, 2006). These hypotheses will need to be re-evaluated for other euendolith as well as in natural communities.”

Regarding the question as to “why” some groups of organisms do bore we will now refer to (Cockell and Herrera, 2008) who reviewed the question nicely.

Reference cited in the answer to referee #2

Cockell, C. S. and Herrera, A.: Why are some microorganisms boring?, Trends Microbiol., 16(3), 101–106, 2008.

Garcia-Pichel, F.: Plausible mechanisms for the boring on carbonates by microbial phototrophs, Sediment. Geol., 185(3–4), 205–213, 2006.

Golubic, S., Campbell, S. E., Drobne, K., Cameron, B., Balsam, W. L., Cimerman, F. and Dubois, L.: Microbial endoliths: a benthic overprint in the sedimentary record, and a paleobathymetric cross-reference with Foraminifera, J. Paleontol., 58(2), 351–361, 1984.

Haigler, S. A.: Boring mechanism of Polydora websteri inhabiting Crassostrea virginica, Am. Zool., 9(3), 821–828, 1969.