

Dear Editor,

Please find attached a reply to the reviewer comments. We thank you and the anonymous reviewer for the thorough and constructive comments and appreciate the time and effort that you have invested in improving this manuscript.

Thank you again for a thorough and efficient review process. We will be glad to answer any further questions.

Sincerely,

Nimrod Wieler

Comments by Reviewer

In the manuscript “Estimating the growth rate in desert biological rock crusts by integrating archaeological and geological records” by Wieler et al., the authors propose a creative method for dating the age of archeological sites by evaluating the growth rates of the rock-crust of biological origin. Although a helpful and required method, the assumptions and constraints made should be better specified.

A: Thank you for this constructive feedback, please see our reply to specific comments below.

R: It is not clear to me whether the studied rocks are defined as desert varnish rocks, or under a broader case of rock crust. On one hand, the authors reference varnished rock studies but do not term them as such. I’m afraid that in some cases, this may mislead the readers. For example, the paper by Lang-Yona et al. does indicate that fungi are scars in type I varnished rocks, but this does not mean that other rock-crusts (or other types of varnished rocks) will have the same microbial composition. Varnished rocks can be clustered into five categories, based on their elemental composition, formation rate, and structure, implying possible differences in their formation mechanisms (Macholdt et al., 2017).

Therefore, a more precise definition of the type of studied rock-crust is needed, in addition to accuracy in citing other types of crust structures.

A: Thank you for pointing out this vagueness. The crusts we studied are hardpan-laminated structures composed of masses of micritic to microsparitic carbonate layers, interbedded with microbial coatings and, in turn, cover the lime and chalk host-rocks (as defined in lines 74-75). Such laminated structures were previously reported by Alonso-Zarza and Wright, (2010) as stage four terrestrial calcretes. Comparison of the microbial community between the different biological rock crusts is mentioned as a base for broader scale of understanding of such rock crusts growth rates. We use the term biological rock crust (BRC) as it was previously coined by Gorbushina (2007) for microbial communities that develop on solid mineral surfaces exposed to the atmosphere. We have clarified this in lines 22-25

R: The authors estimate a general growth rate value for biological rock crust (BRC), based on the chalk and limestone crust thickness and the assumption of crust accumulation starts with the establishment of the site. While the idea is creative and provides a helpful tool for archeological dating, I am not convinced that this equation can be applied to other environments and different types of rock crusts for the following reasons:

- 1. As the results show, the thickness of the chalk crust is 1-fold smaller than that of the limestone (line 72-74), and the thickness is not even over the same rock, as in limestone. This indicates that either the rate of formation is not even over different locations, or that the crust degrades/falls off with time. In the first case, one cannot assume a constant rate of formation, and in the latter case, how can the actual thickness of the crust representing the zero-starting point of crust accumulation can be determined? I presume assumptions have been made here, but they should be clearly stated, in order for this tool to be applied.**

A: Thank you for mentioning it. The BRC thickness on the two lithologies (i.e., chalk and limestone) at the Byzantine site was measured and compared to the chalk and limestone BRC at natural rock outcrops located in the adjacent slopes. As a result, we find the chalk blocks at the Byzantine site as a reliable zero starting point for estimating BRC growth rates (lines 130-132). The thickness of the crust is indeed uneven, to some extent. This is to be expected and is precisely why our measurements provide a range of values for estimating the growth rate of the crusts, rather than a single point estimate.

- 2. The type of stone is only one constrain. Others include the directionality of the stone and exposure to sun radiation, the porosity of the stone, mineral content, humidity, slope directionality, etc. The authors should constrain their proposed rates to hold safe under specific conditions, and as an average rate with possible upper and lower limits.**

A: Thank you for raising this issue. To avoid the slope aspect effect that may lead to different moisture regime, all samples were retrieved from a south-facing slopes/walls

and were collected during the same time period (lines 220-222). To constrain the geotechnical properties of the subjected lithologies we performed different analyses including porosity, rock bulk density and mineralogy (Table 1). Naturally, our growth estimates cannot be translated “as is” to any setting, but must be calibrated to match the local climate conditions and mineral composition. This is now clarified in lines 134-13523: “Calibrating of local climate conditions and mineral composition is needed for BRCs growth rates when applying on different conditions”

3. I am missing a validation test for this method. Did the author sample other sites and tried calculating the age of the stone based on the thickness of the BRC?

A: Thank you for the suggestion. We now conducted a validation to the BRC thickness dating method at a different archaeological site called “Nitzana Byzantine site”. The new site is also located in the Negev Desert, Israel; some 20 km from our original site. Our measured BRC thickness results at this site show a 100-200 μm crusts. This is now clarified in the “Method validation” section (lines 84-88) and in Fig. S2. These findings are consistent with the BRC thickness crusts that were measured at Shivta archaeological site.

R: The statement of bio-crust causing the difference in $d^{13}\text{C}$ between the rock types is thin. The authors do not present analyses of negative control rocks with no bio-crust, to prove that the difference in $d^{13}\text{C}$ values between chalk and limestone indeed comes from the crust activity. Therefore, I am not convinced that this is the reason for the difference, rather than the age of the rock, the structure, porosity, density, etc.

A: Thank you. To confirm the biogenic nature of the BRC upon the two lithologies we conducted isotopic cross section for each lithology separately. This analysis was conducted for BRC's both from the Byzantine site and the adjacent natural slopes. The $d^{13}\text{C}$ profiles compared the crust to their host rock that underlie beneath it. The host rock was used as the negative control for each lithology in the given cross section. We find the limestone BRC $d^{13}\text{C}$ values to show large difference compared to the host rock (lines 91-93, Fig.3A). No similar clear observation was found upon the chalk BRC $d^{13}\text{C}$ values (lines 93-94, Fig.3B). The differences in $d^{13}\text{C}$ values between the chalk and limestone are suggested to result from the BRC thickness. Thicker BRCs, as observed in the limestone samples, may hold more biogenic activity compared to the chalk (lines 97-98).

R: In figure 4C, how does the stone type impact the coordinates of the samples' bacterial composition? This would be a valuable addition of information into the analysis. In addition, PCoA or other such analysis linking different parameters to the microbial composition may also be a valuable addition. For example, the impact of porosity, surface-to-volume ratio of the crust, crust thickness, and other parameters on the distribution of community composition of the samples in the coordinate matrix may give a hint on key microbes' preference under different conditions.

A: As can be seen in Table S2, variance-partitioning analysis using PERMANOVA showed neither a significant effect of the lithology nor of the interaction between the lithology and sample source on the community compositions. Hence this was excluded from the model shown in Fig. 4. This similarity in composition demonstrates the indifference of microorganisms to the type of attachment surface in this case and that the community probably changes very little after establishing. However, the differences in BRC thickness between the chalk and limestone sampled from the slopes could indicate that the latter can better support BRC growth (lines 138-140).