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Interactive comment on "On giant shoulders: How a seamount affects the microbial community composition of seawater and sponges" *by* Kathrin Busch et al.

Anonymous Referee #2

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General evaluation

The paper describes pelagic microbial communities at a seamount and in the surrounding deep sea and compares these communities with sponge-associated microbial communities at the same locations. The authors conclude that biogeochemical properties of the water column and hydrodynamic effects induced by the seamount topography may shape these communities and explain differences between seamount summit, flanks and far field stations. These aspects have rarely, if ever, been investigated at seamounts, and the study is an important and interesting contribution to the knowledge of seamount ecology. Although the paper is generally well written, it lacks

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some important information regarding methodological aspects, the results are not always presented precisely, and the discussion is superficial in parts, with the results not being fully exploited. For example, the samples were taken in three consecutive years, which could have biased the results considerably, but it is nowhere mentioned which samples were taken in which year and how they differed, and interannual variability and its possible consequences are not discussed. The results of the hydrographic measurements are difficult to see; Fig. 3 is not helpful in this respect. The discussion of relative abundances focuses on only one phylum, but the interesting overall patterns are not considered. There are more issues throughout the text; details are given below. I think that a major revision can improve the paper considerably.

Detailed comments

Abstract

Line 28: I think it should read "at least 200 m", since only two depths were sampled L.40/41: explain abbreviations HMA and LMA

Introduction

L46: although seamounts are widely recognised meanwhile, I would suggest to include a definition here L53: "stimulated primary productivity": insert citation here. This hypothesis has rarely been verified

Methods

L100-108: a figure /map of the seamount location should be included here. Figure 2C could be used as an inset to illustrate the bathymetry. More information is necessary of seamount features: base depth, shape L104: Why this isolated presentation of the near-sea bed temperature? The temperature distribution as derived from the CTD should be presented in the Results, including the allocation of water masses (see also below) L105: I do not quite understand what "minimal values" means in this context L111: See also comment in the general evaluation. How were the samples distributed

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across the three years, which subset was taken in which year? This is essential information, since it is well known that temporal variability of water column properties is very high at different scales, and particularly at seamounts with their highly dynamic hydrographic regime. . L113: Again, a map of the sampling locations, including the sponge samples, should be included here, for example based on Fig. 2C. Looking at this figure, all stations were aligned, with some variation, along a W-E axis, with one exception, and I cannot see that any samples were taken along the 74°N-latitude. L117: replace "which" with "and". L117-120: This statistic is strange here. First of all, it is not clear how an ANOVA can be applied to single measurements, but obviously some stations were pooled; the reason and which stations are not provided here (this becomes more clear only later). And even in this case, means and SEs (and hence an ANOVA) make no sense here, because the measurements of depth are no independent replicate measurements of a population, but are just taken at different locations. And, of course, it is trivial that the depth at the summit is shallower than at the flanks and at the base. . . That's how these regions are defined. Here, just the depth ranges should be indicated. L121: Were these sponges sampled at the same time as the water samples? The number of sponges sampled (i.e. four in each BW1-4 as presented later) indicates that a targeted sampling was done in the subareas defined by the microbial clusters of the near-seabed samples; i.e. probably much later. This information is important for the interpretation. L150: see also comment above. Without knowing the results, it is not clear what is meant here as "sampling region". L186: the purpose of this correlation matrix is not clear. It is not dealt with in the discussion.

Results

L192ff: The extrapolations based on "machine learning" in the contour plots of Fig. 2A and 2B appear very arbitrary, e.g. the N-S extension of MW1 in Fig. 2A, or, even worse, in Fig. 2B, where e.g. BW2 and BW3 extend far into a region which was not covered by samples and features different bathymetric (and most probably also hydrographic) conditions - this is highly unlikely. Even BW1, which was obviously found at

only one station, appears to be present also in in patches in the south and in the north. These extrapolations are confusing and also unnecessary for the interpretation of the results. I suggest to omit the extrapolations and show only the station dots with their respective colours indicating the allocations to the clusters. Line 200: see above; Fig. 2C should be presented in 2.1 L203: replace "overlaying" with "overlying". L202-206: Fig. 3 is too complicated, and the additional results (oceanographic setting) cannot be adequately deduced from the figure. I suggest to provide either simpleT/D-plots, or a 2-dimensional contour plot of temperature with a clear indication of water masses along the main sampling axis. The figure may be useful for interpretations, but then in the discussion section L209: this is not quite logical; the exception from the biodiversity in MW being lower than in BW would be a higher (or equal) biodiversity in MW, but not a difference between BW samples. Why are no data presented here like for the overall richness in BW and MW samples? L212: does this apply only to the summit or to all regions? L213: the difference between this analysis and the one before is not clear. What is "pool" in this respect, and how did these differ? L218: be precise: obviously not samples, but sample regions defined by microbial clustering were compared. L224-234: it would be interesting to see which clusters differed from each other in their biogeochemical properties (e.g. pairwise comparisons). Acc. to Fig. 2B, cluster BW1 consists of only one sample; how was this considered in the ANOVAs? L226: "increased with depth" L223: Here, only the summit stations were compared with respect to their biogeochemical parameters. What about the other locations? L248: Was this correlation with depth statistically tested? How? L255: Interestingly, looking at Fig. 6, Protobacteria had a much higher relative abundance in BW1 than in the other clusters, whereas Gemmatimonadetes had much lower abundance in BW1, but in both phyla differences were not significant. Is there an explanation? In this context, it would be very interesting which clusters differed from each other. For example, Fig. 6 suggests that the differences were mainly between BW1 and the other clusters, which showed only small differences. Could this be tested? L258: see also comment in M&M. This information is not further used, and it is hardly or not at all discernible from Fig. 6.

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L264: where is this analysis (correlation between biogeochemical parameters and relative abundances), and how was the statistics done (was this correlation independently tested?)? In Fig. 6, only some relationship between depth and relative abundances is indicated, with differences between depths always corresponding to differences between clusters L266: it is not clear what "significant variability" means in this context, and how this variability was tested L267: which pattern?

Discussion L276: But according to the results (L212ff) community clusters were significantly different between BW and MW samples. This contradiction has to be resolved. L284: Since only one LW depth was sampled, this process could extend far higher than the 200 m, so it should better read "at least". But the process may not have necessarily been restricted to the summit, because due to the much greater distance between LW and BW samples at the other stations, a similar effect may just not have been detected. L291: this applies also to the southern hemisphere! L293: this is far from clear and cannot be deduced from Fig. 3. Apart from the separated clusters at the summit, which may in fact be related to retention and vertical mixing by, e.g., a Taylor column, it is not shown how differences between stations could relate to oscillations of the water column L300: this comparison is hardly applicable here. The Morato et al paper deals with large pelagic predators, and their enhanced biodiversity at seamounts, which is not restricted to the summits, has underlying mechanisms completely different from microbial communities. L307: in which respect do they change? Some information would be helpful (without needing to consult the literature) L310: These are not discernible in Fig. 3. See also comment in the General Evaluation concerning Fig. 3 L312: it is not clear what is meant by "dense ecosystems" L316: include "probably" before "based" - there is no direct evidence L350: "were positively correlated with depth. . ." No correlation analysis was done between biogeochemical parameters and depth, but discrete ANOVAs for each parameter which revealed differences between cluster regions. These appeared to covary with depth. L353: where does this prediction come from? L354: this ("typically carry more oxygen. . .") is redundant to the statement before L361: is it really depth (pressure?) that structures the communities,

or depth-associated parameters? L363-370: Why are only Chloroflexi in G. hentscheli discussed? What about the other phyla and sponge species? Particularly with G. hentscheli, there are some interesting patterns which should be discussed in more detail. For example, whereas the relative abundanes of most taxa are very similar in the BG2-BG4 clusters, the abundance of Chloroflexi and Acidobacteria are much lower in BW1, whereas Protobacteria are much higher - is there any explanation? L363: Acc. to Fig. 6, there is a big difference between BW 1 and the other clusters, which are very similar to each other. Has it been tested which clusters differ from each other? L364: I cannot find any results about a positive correlation between these parameters and the clusters L367-369: this is not quite clear here. Usually, the oxygen demand is enhanced by the (microbial) degradation of OM, which on the other hand sets free nutrients such as NO3- and may enhance denitrification processes. It would be interesting in this respect to learn something about the metabolic pathways of Chloroflexi, e.g. whether they are involved in denitrification, which could explain a positive correlation with NO3-.

Conclusions

L378:"high resolution sampling" is rather meaningless - distributing sampling over three years is rather not high resolution, and whether the spatial resolution is high is also questionable. I suggest to omit this; it is not necessary. 380: "has a detectable but variable influence. . ." I would be careful with this statement. There appeared to be some interrelation (a statistical correlation was not shown), but it could not be convincingly shown that a causal relationship with those parameters was highly likely, or which of the three was probably the key parameter. A possible mechanistic explanation would be interesting, for example with respect to metabolic functioning of the microbial phyla. What about interannual variability - the paper does not provide any information that would rule out a possible effect of the sampling dates.

Figures

Fig. 2: see comments in Results. Fig. 2C belongs into the Methods section

Fig. 3: This Figure should be placed into the Discussion and help interpreting the results. It is not suitable for the presentation of results, because, for example, the temperature profiles and water mass distribution are not readily identifiable in the 3D setting

Fig. 4: y-axis labelling is missing. Degrees of freedom of the ANOVAs should be included.

Fig. 6: the correlation matrix should be omitted - it is not used and is hardly (A and B) or not all discernible (C and D). Panel C: Geodia in italics.

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