

Interactive comment on “Microbial colonisation in diverse surface soil types in Surtsey and diversity analysis of its subsurface microbiota” by V. Marteinson et al.

Anonymous Referee #3

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The study reported by Marteinson et al. is intrinsically intriguing due to the unique study site, well within the scope of the Journal, and yields some valuable insights into microbial colonization and succession in volcanic soils. I therefore have no hesitation in recommending the manuscript for publication in the Journal. Nevertheless, there are some issues that warrant discussion and the authors may perhaps clarify to some extent in the final publication.

Firstly, it is unclear why clone libraries were only obtained from the subsurface samples and not the surface soil samples. This somewhat diminishes their utility. I am also somewhat unclear on the sizes of the clone libraries, which should be reported in either the Methods or Results sections. How many clones were obtained per sample?

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Judging by the Discussion, they seem rather few. Perhaps too few to draw meaningful inferences from on the population structures within the samples? I would urge the researchers to follow up on their study using cloning-free NGS metagenomic or 16S-targeted methods to obtain a deeper understanding of the populations present in the samples.

The authors attempted to obtain enrichment cultures from the subsurface samples. It is confusing that in the Methods section, it says that "growth was confirmed with microscopy" whereas in the Results section it is stated that "no growth could be observed". If the latter is true, the authors should rephrase the former (e.g. "enrichment cultures were examined for growth using ..."). Also, please be more specific in the Results section ("several weeks" ... how many?). And why were the enrichment attempts abandoned after "several weeks"? It is somewhat surprising, given the methodology described, that no growth occurred in the enrichments cultures. The authors may want to speculate on possible reasons for this.

The authors used standard plate counts on R2A and PCA pour-plates at 22°C for 72 h to estimate the total viable counts. A justification for this choice of methods should be presented. Of course, no single (or even a few) culture conditions will ever yield a truly "total viable" count, but I wonder, given the environmental conditions expected in Surtsey, if lower temperature/longer time/lower nutrient concentration spread-plates should perhaps have been considered? Similarly, the choice of target organisms (coliforms, particular pathogens, ...) is a little perplexing. Although they make good sense in relation to the bird droppings, one would have liked to see also included other, more biogeochemically relevant focus organisms. Many of those (e.g. comamonads, oxalobacteria, the various actinobacteria, pseudomonads, sphingomonads ...) are readily culturable and could have been included in a similar fashion. Nevertheless, the results of the culture-based studies are intriguing and appear carefully and competently analyzed. Indeed, the multivariate analysis appears to yield some intriguing results that would merit a more in-depth discussion. In fact, the Discussion section on the whole

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could do with an expansion.

Finally, there are some minor language-related issues and a couple of typos and other minor glitches that should be fixed. E.g., in Abstract line 4: "have been focusing" should read "have focused", Abstract line 11: can 182 m really be considered "the deep subsurface"?, there are several instances throughout the manuscript of "despite of" – please delete the "of", Section 2.1.2 line 24: please specify the number of days at 30°C, section 3.1.4 line 4: why is "data not shown"?, Section 4.1 line 24: "showed" should be "shown", line 22 "between" should be "among".

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