This study is a compelling analyses of omics data and nickel isotope fractionation data in a first attempt to find microbial communities in relation to nickel fractionation in the Southern Ocean. As an applied bioinformatician with a theses background on Southern Ocean microbiology I can mostly comment on the methodologies concerning data analyses of omics data. However, I would foremost like to address the difficulty of linking Nickel to Nitrogen fixation or urease activity when the paper used no data for inorganic nitrogen nor urea. It is solely trying to reconcile gene family clusters and nickel fractionation data, which can be an interesting attempt however it leaves the impression that they were fishing in the data to find links with Nickel. I do think that these attempts are important however the study design would have benefitted from sampling for parameters that would make the interpretation of these results easier. Besides this is a well done and neat study of high quality. All the files are carefully curated the dataset it accessible and the majority of the figures is very well explained.

Detailed comments below:

Introduction

First paragraph introduction. Focusing on Ni-SOD which present in Cyanobacteria an Ostreococcus both not found in significant abundance in Southern Ocean waters.
67 – Define biogeochemical divide
70-80: Very loose ends tried to be stitched together. Diazotrophs also very rare to be found in Southern Ocean dataset even if potential is there. Inorganic N not necessarily
80-92: Very long section on metagenomics which could be partly shortened or moved to methods. Depending if Biogesciences readership needs this long introduction on the methodology. But I do not think it is necessary to make it this long.
85: Sequenced metagenomic samples. But the sentence needs rewriting. Short DNA fragments are reads can be defined in the first sentence
93: Bio-mediated process is vague. And the paragraph does not explain why metagenomics is the ideal tool for investigation.
103: The coupling of the different dataset would need a paragraph in the discussion maybe on scales and how the scale differences are addressed as seen here https://www.nature.com/articles/nmicrobiol201528
105: There is no clear hypothesis or clearly stated objectives in the introduction which is often the case with hypothesis-free driven omics exploration. I think the authors could try to clarify this in the introduction. However, I would be careful with 'enzymatic needs' as there is no rate measurement and this cannot be derived from omics data.

Material and methods

130: I do not find the exact depths at which sea water samples were taken for omics data exploration. What were the surface water depths? Mixed layer depth? Deep chlorophyll maximum?
& 180: It is also not clear which were the same stations for Nickel values and omics size classes used further on. Figure 1 shows the Nickel and isotope samples, were the omics samples taken at the same stations?
190: past tense, tools were used. It seems as if this is an introductory sentence, not sure if it needed. The tools have no versions but they are described later. Could be removed.

205-215: Did you try checking on Expazy for metal related enzymes? You could have also tried to use the nickel co-factor enzymes. However, the question whether higher presence of metal related genes indicated higher need or utilization will remain.
220: Going back to the introduction you focus on enzymes related to nitrogen metabolism and alternative nitrogen fixation in an area of the ocean where little measurable nitrogen fixation is taking place.
230: Have you tried matching against
https://academic.oup.com/ismej/article/15/10/2933/7474411
https://www.nature.com/articles/ismej201231
https://pubmed.ncbi.nlm.nih.gov/23126454/
https://pubmed.ncbi.nlm.nih.gov/22806143/

Results:
305: By a quick research I see multiple enzymes contain nickels, hydrogenase, and CO-dehydrogenase. What about NikR, nickel sensor enzymes? Did the authors check for these? could you add the amount of Ni in these three enzymes? Are there any quota available such as for Fe rich enzymes? Or Ni quota for prokaryotic cells in general?
The main result here is that in correlation with Ni this is difficult to interpret but enzymes that also contain nickel seem to be correlated to some extent for small or large size classes. I was surprised that the authors do not give information on the taxonomy behind these clusters (AGC) and abundance of microbial communities.

Discussion:
357: explain how this is consistent with the biogeochemical divide
430-445: but how do you link this to existing literature and information to these heterotrophic bacteria?
451: Difficult to know without urea measurements
453: Can we really define this as urease activity? Activity of the enzyme was not measured just presence of contigs and mapping of reads.
What is the cellular Ni requirement?
475: Are there studies showing that Rhodobacter or alphaproteobacterial use urea based nitrogen? They are usually found in bloom nutrient rich areas where NH4 is abundant.