Dear Editor and Referee,

We first would like to thank you for your constructive review. All suggestions have been considered and used to improve our manuscript entitled "The Ni cycle in the Southern Ocean: insights from Ni concentrations, isotopes and metagenomics".

Please see our detailed answers, including corresponding lines of the revised manuscript, in the attached pdf document. In this document, the comments of the reviewers are in *italics*, followed by our answers in blue font. The new text that we propose to add to the revised manuscript is in red font.

We hope that you will find the revised manuscript suitable for publication.

Regards,
Nolwenn Lemaitre, Emile Faure and co-authors

Response to the review submitted on 09 June 2026:

- *Given this hypothesis, it makes sense to look at the correlation between nitrogen and Ni concentration or isotope. The authors have a short paragraph on this (L366-371), but it does not cite any figure (I assume Figure 8 should be cited. In that case, you want to adjust the figure order as this figure shows up earlier than Figures 5 - 7).*

We now cite Figure 5 (previously Figure 8) in the Results section describing the PCA.

Lines 382-384: The first axis of the PCA achieved on the compilation of Ni and nitrogen variables explained 54.2% of the variance, and opposed samples with high nitrate, NOx and Ni concentrations, with samples showing high concentrations of PON, $\delta^{60}Ni$ and $\delta^{15}N_{PON}$ (Figure 5).

- *I suggest also adding regression analyses (like Figure 3) between nitrogen and Ni concentration/isotope in addition to the PCA analysis.*

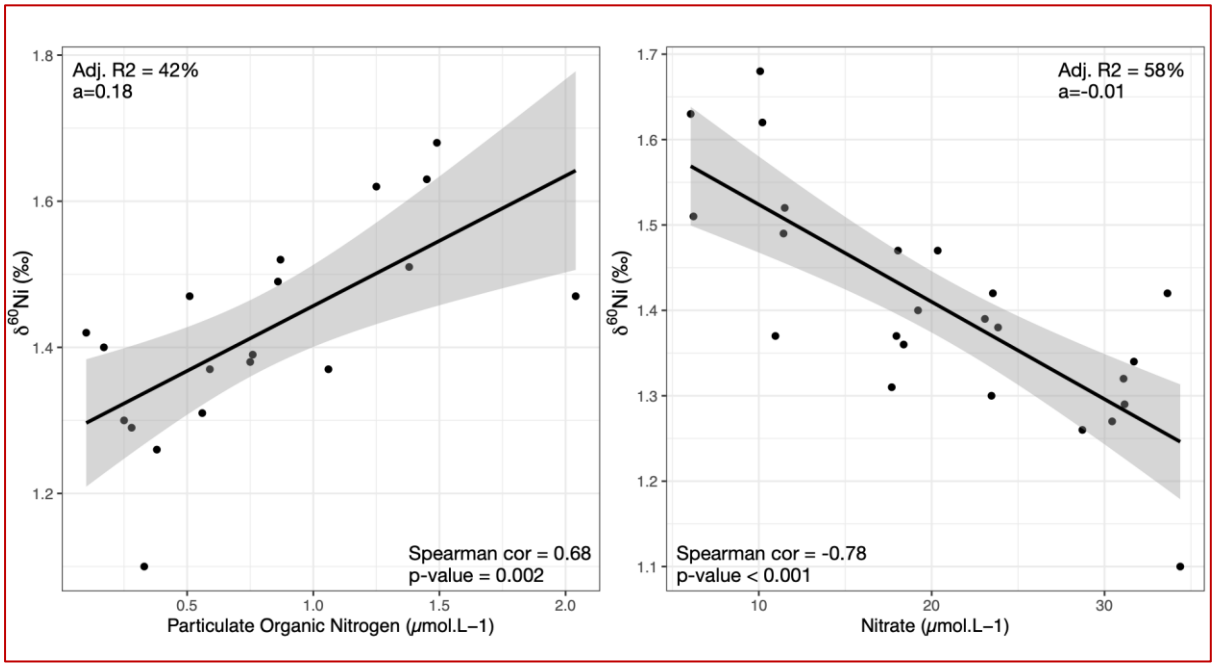
We now provide regression analyses as Figure S5 in the Supplementary Information. These regressions reinforce the links between $\delta^{60}Ni$ and nitrate as well as PON concentrations.

Lines 293-295: To complement this PCA we further computed individual correlation analysis of the relationship between $\delta^{60}Ni$ and PON and nitrate concentrations, again using the rank-based Spearman coefficient and a two-sided permutation-based testing approach.

Lines 384-385: The correlations between $\delta^{60}Ni$ and PON and $\delta^{60}Ni$ and nitrates were both significant (p-value = 0.002 and < 0.001, respectively; Figure S6).

Figure S6: Correlation analysis of the relationship between Ni isotope composition ($\delta^{60}Ni$) and particulate organic nitrogen (PON; left panel) and nitrate (right panel) concentrations. Each dot represents a sample, spearman correlation coefficients and their corresponding p-values are

shown at the bottom of each plot. A regression line surrounded by a 95% confidence interval plotted in grey is represented for each plot. The corresponding adjusted R2 and coefficient are shown at the top of each graph.



- *Another suggestion related to this is that this center hypothesis could be flashed out a bit more early on. Especially for the study area, I suggest introducing this hypothesis as well as how iron and other stressors might limit the nitrogen uptake by phytoplankton, and limit nitrogen fixation (the requirement of Fe by nitrogenase).*

We thank the reviewer for this comment. We now describe this hypothesis in the introduction by adding a new paragraph before introducing the isotopes, and we have modified the paragraph on the third hypothesis related to the biogeochemical divide.

Lines 60-69: This link between both cycles may be reinforced in low-latitude oligotrophic surface waters where dissolved inorganic nitrogen is often limiting, thereby increasing the importance of alternative nitrogen sources such as urea and atmospheric dinitrogen. The acquisition of both nitrogen sources relies on the Ni-containing enzymes urease and NiFe hydrogenase, the latter protecting the nitrogen fixation process and producing energy that subsequently supports nitrogen fixation (Li et al., 2022). The Ni-SOD enzyme also plays an important role for diazotrophs as it has been shown to protect the nitrogen fixation process. Specifically, the 'nitrogenase' enzyme, responsible for the dinitrogen fixation process (nif genes) and requiring iron as a cofactor, is vulnerable when exposed to reactive oxygen species (Chen et al., 2022). Moreover, high light conditions and oxidative stress in low-latitude surface waters may also increase Ni requirements for the utilisation of the Ni-SOD enzyme. Together, these processes could increase Ni requirements in low-latitude surface waters. Understanding microbial Ni utilisation is therefore essential to interpret global Ni distributions in the ocean.

Lines 89-94: In this study, we aim at testing this third proposition and whether Ni could have a special role in the microbial metabolism of low-latitude regions, where its utilisation is essential in urea acquisition, oxidative stress protection and nitrogen fixation (e.g., Price and Morel 1991; Chen et al., 2022; Li et al., 2022). In fact, heavier surface Ni isotope compositions were found to correlate with higher nitrogen fixation rates in the North Atlantic (Lemaitre et al., 2022). However, because of the different enzymatic roles in nitrogen metabolism, disentangling the specific process (or processes) leading to low-latitude surface water Ni depletion and isotope fractionation is challenging.

- *Another suggestion is that the small and large size fractions are not really introduced. Why are these two size fractions sampled? What microbes from the study area do you expect to be primarily associated with small or large fractions, and why does this matter for Ni?*

Thank you for pointing this out. We provide more details about the two size fractions in the Method section where they are firstly mentioned.

Lines 199-202: The small size fraction is dominated by bacteria and archaea, likely mostly free-living, while the large size fraction is dominated by unicellular eukaryotes, but also contains particle-attached organisms (Faure et al., 2026). This way, Ni-containing enzymes from phototrophic and heterotrophic bacteria are likely to be found in the small size fraction, while enzymes from eukaryotic micro-algae like diatoms will mostly be limited to the large size fraction.

- *Title: the current title is a bit stronger than the evidence presented. I would revise it to something like: "The Ni cycle in the Southern Ocean: insights from Ni concentrations, isotopes and metagenomics".*

We have modified the title accordingly.

- *L 33 Ni-SOD should be spelled out.*

This was modified accordingly.

- *L 35 their and L 39 of should be fixed.*

These were corrected.

- *L 60-61: please explain what "very low concentrations" means. 1.7 nmol/L to readers unfamiliar with trace metal biogeochemistry is very low concentration.*

We agree that the term "very low concentration" is relative and may not be meaningful to readers unfamiliar with trace-metal biogeochemistry. The sentence has been modified.

Lines 71-75: Despite these important biological demands, Ni concentrations are never fully depleted in the surface ocean and reach a ubiquitous minimum of around 1.7 nmol/L in the

surface ocean (GEOTRACES Intermediate Data Product Group, 2025). Because of this incomplete drawdown, Ni has generally not been considered as a limiting nutrient in the ocean – unlike several other bioactive trace metals that can be depleted to near zero concentrations in surface waters – and consequently Ni remains under-studied.

- *L 78: the latter proposition would be clearer if you had introduced two hypotheses only. However, three were introduced. I would make it more specific about which hypothesis you are referring to.*

We provide clarity on the tackled hypothesis.

Lines 89-91: In this study, we aim at testing this third proposition and whether Ni could have a special role in the microbial metabolism of low-latitude regions, where its utilisation is essential in urea acquisition, oxidative stress protection and nitrogen fixation (e.g., Price and Morel, 1991; Chen et al., 2022; Li et al., 2022).

- *L 80: please be specific about the correlation, such as: "heavier surface Ni isotope was found to correlated with larger nitrogen fixation rates …" (this is only an example, please make sure it reflects what was shown in the study)*

This was modified accordingly.

Lines 91-92: In fact, heavier surface Ni isotope compositions were found to correlate with higher nitrogen fixation rates in the North Atlantic (Lemaitre et al., 2022).

- *Methods: SOD enzymes do not necessarily require Ni (L50-53). Does your gene annotation include all SOD or only Ni ones? If the former, is there a way to screen for Ni containing SOD only? If the latter, please provide additional details of this screening in the Methods section.*

All types of SOD were retrieved by our approach, as some levels of functional annotations do not allow to distinguish them (*e.g.*, "*Superoxide dismutase*"). Still, by using more specific annotations like the PFAM ones we are able to distinguish Ni-SOD. More details are now given in the Method section.

Lines 233-235: Please note that all types of superoxide dismutase were retrieved in our selection, and we provide results both at the level of the whole enzymatic family (*e.g.*, "*Superoxide dismutase*" eggNOG description) and focusing more specifically on the Ni-SOD (*e.g.*, "*Sod_Ni*" PFAM annotation).

- *Figure 4 and Figure 8: could you be more specific about what each dot mean in these two figures? Either in the figure captions or in the Methods section: it would be helpful to include statements such as: "each dot represents a sample from a specific depth at a station, calculated from a matrix containing …".*

The captions of Figure 4 and 8 (now Figure 5) have been modified to be, hopefully, clearer.

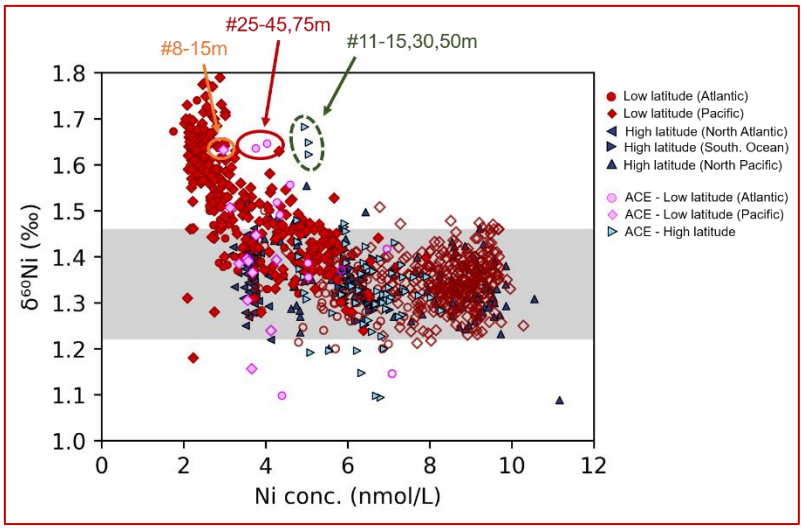
Caption of Figure 4: Redundancy analyses (RDA) on the rCLR-transformed abundance of AGNOSTOS gene clusters (AGC) and Ni distributions (concentrations and isotope compositions) for a) the small size fraction (0.2 - 3 µm and 0.2 - 40 µm; on the left side) and b) the large size fraction (3 - 200 µm; on the right side). Each plain arrow corresponds to a gene cluster. Light red arrows correspond to AGC unrelated to δ^{60}Ni, orange arrows to δ^{60}Ni-related AGC more abundant in stations 8 and 25, yellow arrows to δ^{60}Ni-related AGC well represented in all high-δ^{60}Ni stations, and dark red arrows to δ^{60}Ni-related AGC more abundant at station 11. **Each blue dot represents a sample from a specific depth, with the station number shown in white in the middle of the dot, and with the colour indicating the δ^{60}Ni value. Positions of the dots reflect both the enzymatic and Ni/δ^{60}Ni systematics in each sample.** Taxonomic and functional annotations of these gene clusters are given in Tables 1 and S2.

Caption of Figure 5: Principal Component Analysis (PCA) on the Ni concentrations and isotopes (δ^{60}Ni) together with inorganic nitrogen concentrations (nitrate, nitrite, ammonium, NOx), particulate organic nitrogen (PON) concentrations and nitrogen isotope composition from PON (δ^{15}N). Similarly to Figure 4, **each blue dot represents a sample from a specific depth, with the station number shown in white in the middle of the dot, and with the colour indicating the δ^{60}Ni value. Positions of the dots reflect the N-related and Ni-related variables of the different samples.**

- *Figure 5: what do the circled numbers 1 – 4 in the figure represent? Please clarify this in the caption.*

Thank you for noting this. The circled numbers represented endmembers from different oceanic basins, as done in Lemaitre et al. (2022). However, we decided to not discuss them as they do not bring essential information in this new study. They have been removed from the new figure.

Figure 6 (previously Figure 5): Comparison of the new ACE dataset with the dissolved Ni-δ^{60}Ni systematics of the global ocean (Cameron and Vance, 2014; Takano et al., 2017; Wang et al., 2019; Archer et al., 2020; Yang et al., 2020, 2021; Lemaitre et al., 2022; Bian et al., 2024; Chiu et al., 2026; and this study). The shaded grey band shows the deep-ocean δ^{60}Ni value (see Figure 2).



- *L302: "As for Ni concentrations": should this be Ni isotopes instead? The following paragraph is about Ni isotope.*

The sentence intended to compare the similar depth profiles of both Ni concentrations and isotope compositions in high latitudes, which remain constant throughout the water column. We apologize for the lack of clarity and have revised the sentence accordingly.

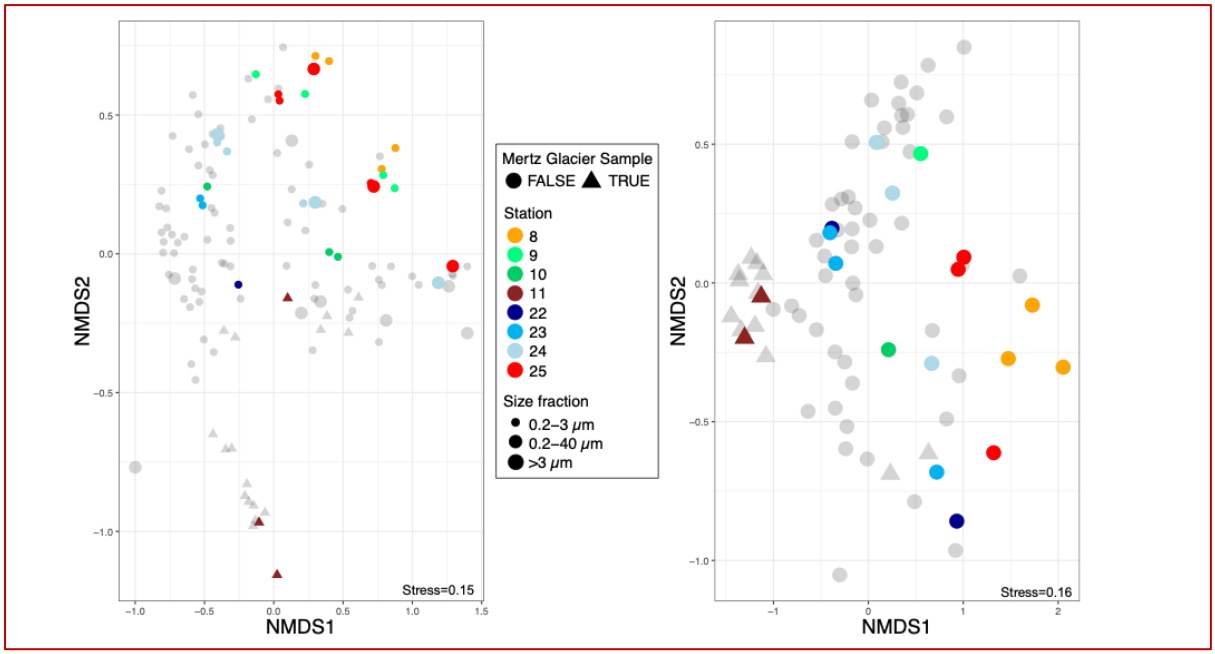
Lines 317-320: Similarly to Ni concentrations, variations in δ^{60}Ni throughout the water column are small at high-latitude stations, usually within the range of the average deep ocean value (1.34 ± 0.12 ‰ at depth > 500 m, mean ± 2SD, shaded band in Figure 2; Cameron and Vance, 2014; Takano et al., 2017; Wang et al., 2019; Archer et al., 2020; Yang et al., 2020, 2021; Lemaitre et al., 2022; Bian et al., 2024; Chiu et al., 2026).

- *L319: "likely because of the strong differences in microbial communities among stations 8, 25 and 11". Since the metagenomes are available, I wonder whether the authors could show some microbial data or cite previous work to support this statement.*

We now provide the reference and a new figure in the Supplementary Information to highlight the different microbial communities among the three stations.

Lines 333-335: Sorting abundances into SOD, urease or NiFe hydrogenase functional groups did not lead to significant correlations with δ^{60}Ni, likely because of the strong differences in microbial communities among stations 8, 25 and 11 (Faure et al., 2026; Figure S5).

Figure S5: Differences in microbial communities across the 8 stations investigated. Code and data from Faure et al. (2026) were retrieved and used to reproduce the non-metric multidimensional scaling (NMDS) of the 218 ACE metagenomes, computed on the abundance matrix of AGNOSTOS gene clusters in the small (left panel) and large (right panel) size fractions. The 48 samples used in this study (*i.e.,* for which Ni concentrations and isotope compositions were available) are represented in colours, while the rest of the ACE samples are shown in grey. Colours indicate the station of sampling, the size of dots matches the size-fraction, and the shape distinguishes Mertz glacier samples (*i.e.,* station 11 represented by triangles) from the rest (represented by dots). Positions of the symbols reflect their composition in gene clusters.



- *L445-447: Table 1 should be cited here if this sentence refers to it.*

The reference to Table 1 has been added.

Lines 461-464: The taxonomic annotations of these clusters indicate that the Ni-SOD is carried by diverse heterotrophic bacteria (*Opitutae, Planctomycetaceae, Piscirickettsiaceae, Alteromonadaceae, Porticoccaceae*) or by *Phaeocystis*, which is a common phytoplanktonic eukaryote across the Southern Ocean known to use Ni-SOD as an antioxidant (Table 1; Tan et al., 2016).

- *L558: in addition to the raw metagenomics data published in another paper, the Ni related contigs, their metadata, and relative abundances specific generated in this study should also be made available. Please provide information on their availability too.*

We have double-checked and this information is available on the figshare link given in the 'Data Availability' section. Please note that this Figshare repository (doi: 10.6084/m9.figshare.29127848) will remain private until acceptance but is already available for editors and reviewers at https://figshare.com/s/d2db1ff17f01b510e012. We have modified the data availability statement to make it clearer.

Lines 574-579: Metagenomic assemblies used in this study are retrievable from from Sextant (https://sextant.ifremer.fr/record/9c786963-e6a5-4a1c-95f8-1c6ab8a52d2b/), while the gene catalogue used in this study is available from Zenodo (https://zenodo.org/records/14181291). All genomic data specific to nickel-related genes (IDs, contigs of origin, taxonomic and functional annotations), all abundance matrices and environmental metadata used in this study are available from the doi: 10.6084/m9.figshare.29127848, as well as tables summarizing quality-filtering and assembly steps statistics.

- *Table S2 is in very low resolution in the PDF version, please make sure the revised version is readable.*

We apologize for the low quality of our tables in this pdf. We now provide an Excel file for each of our tables.