Dear Editor and referees,

We would like to thank you for your useful and positive comments. All suggestions and questions have been considered and used to improve our manuscript entitled "Influence of Ni-related enzymes on the Ni cycle in the Southern Ocean: insights from 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

## 1. Referee 1

- *I do think there are some "negative results" that could be better thought-through that are likely important (like the hound that didn't bark…). Perhaps the authors could explore these implications further? For example, the lack of nitrogen fixers is interesting; why not apply the metagenomics to nitrate/nitrite, or some well-understood nutrient, first? Wouldn't this inform us on the microbial consortium better than jumping straight to Ni?*

  It is a very good point. In fact, Emile Faure and co-authors published recently on the global metagenomics data across the ACE transects, investigating relationships between the genomics and microbial communities and a vast panel of environmental variables, including some related to nitrogen (Faure et al., 2026). We added details in the section 4.2 about their main results in order to inform on the microbial consortium before investigating the Ni-containing enzymes.

  Lines 425-433: Faure et al. (2026) investigated 218 metagenomes across all ACE transects, showing that the Southern Ocean is populated by diverse microbial communities which are largely endemic and structured according to water masses. In addition to characteristics of water masses (temperature, oxygen, salinity and density), they identified the best drivers of microbial composition to be the nitrogen availability (nitrate, NOx) and presence of diatoms (biological silica, silicic acid). They further highlight the strongly contrasting Mertz polynya (station 11), the only coastal Antarctic ecosystem sampled during the ACE cruise, within Antarctic surface waters. Mertz samples were notably enriched in genes involved in organic matter consumption from species typically associated with polar phytoplankton blooms (e.g., ASP10-02a). It is thus clear that station 11 is distinct from stations 8 and 25 (both in subtropical surface waters) in terms of microbial composition, despite all presenting heavy Ni isotope compositions.

  We also propose a new analysis and figure showing a clear link between Ni isotope composition and the nitrogen cycle, at both stations 8, 25 and 11 (Figure 8). The PON concentrations and $\delta^{15}N_{PON}$ values are both high across stations 8, 25 and 11, while nitrate concentrations are at

the lowest at these three stations. This supports the idea of a fractionation of Ni due to its biological utilization by microorganisms facing strong competition for nitrates and using urea as a N source instead. This new analysis supports our enzyme-level results from metagenomics data, which further show that different organisms and potentially even different enzymes could be responsible for this biological use of Ni at the three stations, likely due to strong differences in the primary producers at play at the three sites.

Lines 481-488: Stations 8, 25 and 11 are all characterised by the lowest nitrate concentrations (≤ 10 µmol/L; Table S1, Figure 2) and the highest particulate organic nitrogen (PON) concentrations. Interestingly, the organic matter at these 3 stations is also characterised by elevated nitrogen isotope compositions ($\delta^{15}N_{PON}$), consistent with strong nitrate consumption (Fawcett and Forrer, 2020; Stirnimann et al., 2024). We showed that Ni isotope compositions correlate with PON concentrations as well as $\delta^{15}N_{PON}$ and are inversely correlated with nitrate concentrations (Figure 8). This suggests that enhanced primary productivity at stations with high $\delta^{60}$Ni in surface waters had led to the accumulation of a large PON pool by strongly consuming nitrate. Because microorganisms may face competition for nitrogen acquisition, microbial productivity, particularly bacterial, may thus rely on alternative nitrogen sources, such as urea.

- *When not in a HNLC environment (e.g., the polynya in full bloom), the Ni does fractionate significantly. If biologic fractionation of Ni predicated on limitation, would you expect to see any metagenomics expressed in this HNCL region?*

  Our results show a significant presence of genes coding for Ni-containing enzymes at stations 8, 25 and 11, demonstrating the potential of Ni utilisation by microorganisms in these waters. The activation of these enzymes is expected to remove Ni from the dissolved pool and may induce the observed heavy isotope composition, through preferential uptake of light Ni, as proposed in this study. As the reviewer mentions, the observed high $\delta^{60}$Ni values could reflect a residual, isotopically heavy, organically complexed Ni pool (between 0 and 2 nmol/L), assuming negligible fractionation during biological utilisation (Archer et al. 2020). However, surface Ni concentrations at the ACE stations with high $\delta^{60}$Ni (2.97, 4.95, and 4.34 nmol/L) substantially exceed this range, making it unlikely that a small residual pool dominates the isotope signal. These relatively high concentrations instead support a scenario in which Ni isotope fractionation is linked to biological utilisation via enzymatic processes. This interpretation is further discussed in Section 4.3, and we emphasise the need for additional constraints on the bioavailability and isotope composition of the surface organically complexed Ni pool to better distinguish the mechanisms.

  Lines 517-525: More investigations are needed to thoroughly define the driver(s) of Ni isotope fractionation in the surface ocean. Elevated $\delta^{60}$Ni in surface waters could reflect the preferential biological uptake of light Ni for enzymatic needs, as proposed in this study and in Lemaitre et al. (2022). Alternatively, elevated $\delta^{60}$Ni could come from a residual, isotopically heavy, non-bioavailable Ni pool due to Ni complexation with organic ligands (between 0 and 2 nmol/L) assuming negligible isotope fractionation during biological utilisation (Archer et al., 2020). However, at ACE stations with elevated $\delta^{60}$Ni, surface Ni concentrations (2.97 nmol/L at

station 8, 4.95 nmol/L at station 11 and 4.34 nmol/L at station 25) exceed the threshold of 2 nmol/L, arguing against the dominance of the non-bioavailable pool and instead supporting fractionation associated with biological uptake. We nevertheless advocate for further constraints on the bioavailability and isotope composition of the surface organically complexed Ni pool to distinguish these mechanisms.

- *Moreover, if the fractionation of Ni is closed-system Rayleigh-type (from figure 6), how much depletion of the reservoir is represented.? It looks like Ni never goes lower than 2 nM, even at station 11 (or anywhere according to figure 5). But the upwelled water looks to be all similar at 7 nM and 1.3 permille. More insight in how Ni fractionation could be so different in their sites (figure 6) in conditions that seem geochemically similar (figure 2) would be interesting.*

  We initially discussed the difference in fractionation factors in section 4.1 (lines 400-402) but we added some more discussion about it in section 4.2.
  We also quantified the proportion of Ni removed by biological uptake, as described by a closed-system Rayleigh-type fractionation (see equation in the caption of Figure 6). The theoretical remaining fractions of Ni in surface waters is of 75%, 68% and 59% (corresponding to removal fractions of 25%, 32% and 41%) for stations 8, 25 and 11 respectively. The measured concentration in surface can be explained by such isotope effect, with offsets of less than 0.3 nmol/L after multiplying the remaining fraction to the measured deep concentration. So, the fractionation of Ni is closed-system Rayleigh-type.

  Lines 473-477: Biological uptake by different microorganisms may thus be responsible for the Ni isotope fractionation, with microorganisms specific of the Southern Ocean at station 11, and more widely distributed microorganisms at stations 8 and 25. This geographical difference in microbial populations bearing Ni-containing enzymes may explain the different fractionation factors – and therefore the different proportions of Ni removed by biological uptake – observed among the three ACE stations with high $\delta^{60}$Ni values in surface waters (Figure 6).

- *Here, negative metagenomic results are very impactful, and would seems to point to a component of dissolved Ni in the water that is complexed (unavailable). In light of this, I think the authors really need to address the Archer et al., 2020 and John et al., 2022, 2024 hypotheses described at the start of the ms.*

  This has been developed in section 4.3. Please see our answer to your comment on Ni limitation.

- *The authors should remove station 11 at the outset and then from all statistical analyses, as they make it abundantly clear that it was not typical for the Southern Ocean. There is a lot of leading the reader down a dead-end trail with the discussion including and not including station 11.*

  We have now separated paragraphs discussing stations 8 and 25 from those discussing station 11. To do so, many changes have been made in section 4.2. We understand the point of view of

the referee, as indeed removing station 11 from some analysis but not others can make our results hard to follow. However, we do believe that station 11 is typical of coastal blooming conditions in Antarctic surface waters, which are not negligeable at the scale of the Southern Ocean. The only analysis where we decided to present and discuss results without station 11 is presented in Figure 3. It makes sense to us that most of the statistical signal drawn by station 11 comes from the large size fraction (ecosystem dominated by large diatoms and their associated prokaryotes), while only the small size fraction is relevant for station 8 and 25 (no blooming eukaryote, diversity of primary producers including both Phaeocystis and Cyanobacteria). We find that this justifies our idea of removing Mertz samples in the analysis of the small size fraction, but not in the large one. In all other analyses we find the station 11 to be well integrated, especially as we show now that there are common features related to nitrogen availability at stations 8, 25 and 11 that could explain the high $\delta^{60}$Ni values in surface waters. We thus decided to keep the station while clarifying the results and discussion regarding its treatment in the linear regressions of Figure 3.

Lines 328-329: Station 11 (Mertz polynya) samples drove most of the statistical signal in the large size fraction, while they showed systematically low values of relative abundances in the small size fraction (Figure 3).

Lines 458-459: In contrast of stations 8 and 25, δ60Ni at station 11 seems to be related to large or particulate-attached microorganisms, as shown by the significant co-variations only observed in the large size fraction.

- *Station 11 should be treated as a separate experiment to answer broader questions (e.g,. is a polynya diatom bloom different than "normal" ocean diatom blooms? Why would the microbes, or Ni, be different in a polynya? Certainly there is nothing that would make station 11 stand out when looking at the Ni and NO3- data presented.)*

  That is a very interesting comment! We developed this discussion in section 4.3 by comparing the ACE polynya data to those from the Kerguelen Plateau where intense diatom blooms are also developing and where Ni isotope compositions have been determined. Combined to our new analysis of nitrogen data, this comparison supports our hypotheses on the links between the Ni and nitrogen cycles.

  Lines 497-502: This specific nitrogen biogeochemistry in polynya waters could also explain the surprising heavy Ni isotope composition at the surface of station 11 compared to other high-latitude stations. In the high latitudes, microbial growth is typically supported by high nitrate concentrations from upwelling or nitrification processes (i.e., oxidation of ammonium to nitrite and nitrate). For example, in waters from the Kerguelen Plateau, nitrification has been shown to supply up to 50% of the total nitrate uptake in the mixed layer, thereby preventing any nitrogen limitation (Cavagna et al., 2015; Dehairs et al., 2015; Fripiat et al., 2015). Interestingly, no Ni isotope fractionation was observed in these same Kerguelen samples (Wang et al., 2019).

- *Similarly, there is a lot of method and results text describing the metagenomics (someone with expertise should review this). I understand it is a lot of work, but the description is excessive*

*in comparison to the conclusions drawn from these data. Particularly when excluding the station 11 data. All the various statistical analyses could not conclusively point to a mechanism for the latitudinal variance: why prolong this discussion? And if the results are distinctly negative, this is important, but could be presented quite readily I would think. I think that they do infer negative results, but these points were lost to me in the noise of the statistical hoops trying to get a positive result. (I admit the analyses and statistics done are impressive – I had to look up what a redundancy analysis is – but they seem gratuitous.)*

We took into account this comment and simplified the Methods and Results sections related to metagenomics data. These sections are now shorter. We further separated the methods related to the statistical analyses to make them easier to follow, creating a new sub-section "Statistical analyses and metadata collection".

Regarding the inconclusive aspects of our statistics, we were careful to clearly state the negative aspects of our tests in the results (Lines 318-321: Sorting abundances into SOD, urease or NiFe hydrogenase functional groups did not lead to significant correlations with δ60Ni, likely because of the strong differences in microbial communities among stations 8, 25 and 11. However, positive correlations could be observed for SOD in the small size fraction and urease in the large size fraction (Figure S3).), and the conclusion (Lines 533-535: Our data reveal correlations between enzyme abundance and isotope fractionation, but the strength and significance of these relationships varied with functional databases and stations.).

We do agree with the reviewer that our results at the enzyme-level are overall negative, however we find that our analyses at more precise functional levels and at gene-cluster level illustrate well how this could be due to the huge differences in microbial communities at station 11, 8 and 25. Our new analysis of nitrogen-related variables further pushes towards a link between nitrogen availability and Ni isotope fractionation due to enzymatic processes. We thus find it even more justified now to not stop at the coarse picture of insignificant relationships between enzymes (sums of relative abundances from millions of various genes annotated as multiple distinct parts of each enzyme of interest) and $\delta^{60}$Ni, but to dive into more biological complexity behind these processes.

- *In summary: the authors collected a large and interesting data set, with some unfortunate oversights. They did a great job in presenting the work done, although a lot could be trimmed without losing any of the conclusions. I offer some thoughts above that the authors may consider in revision, but generally I see no logical flaws in their work and recommend this for publication. [with just one silly grip: I read many studies claiming to be "the first time" doing something. While maybe justified, the statement is ostentatious.]*

  We thank the reviewer for this very positive comment. We have trimmed our text, which hopefully will help to highlight our results. We also removed all claims about this study being "the first". We still mention that this study is 'an initial exploration' (line 40) as we feel this statement stands.

**2. Referee 2**

- *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.*

  We thank the reviewer for this comment, which pushed us to go back to the publicly available metadata from the ACE campaign to gather all available information on organic and inorganic nitrogen. We now propose a new analysis and figure showing a clear link between Ni isotope composition and the nitrogen cycle, at stations 8, 25 and 11 (Figure 8). Unfortunately, urease concentrations were not determined during the campaign, which – we agree – would have been highly valuable. The idea of this study emerged long after the ACE cruise, as we wanted to use metagenomics to elucidate on Ni isotope fractionation as well as explore potential biological explanations behind the unsuspected isotope signal at Mertz. The experimental set-ups for the sampling of metagenomics and Ni were thus designed independently, which prevented us to use the full potential of both approaches, and to have a perfectly adapted set of metadata. Still, thanks to the referee's comment, we now provide N-related variables taken from the same CTD samples, which we think add a lot of strength to our hypothesis. The PON concentrations and $\delta^{15}N_{PON}$ values are both high across stations 8, 25 and 11, while nitrate and ammonium concentrations are at the lowest at these three stations. This supports the idea of a fractionation of Ni due to its biological utilisation by microorganisms facing strong competition for nitrate, and using organic nitrogen as N source instead. Our metagenomics data further highlight the fact that different organisms and potentially even different enzymes could be responsible for this biological use of Ni at the three stations, likely due to strong differences in the primary producers at play at the three sites.

  Lines 480-515: 4.3 Implications for global Ni isotope variability
  Stations 8, 25 and 11 are all characterised by the lowest nitrate concentrations (≤ 10 µmol/L; Table S1, Figure 2) and the highest particulate organic nitrogen (PON) concentrations. Interestingly, the organic matter at these 3 stations is also characterised by elevated nitrogen isotope compositions ($\delta^{15}N_{PON}$), consistent with strong nitrate consumption (Fawcett and Forrer, 2020; Stirnimann et al., 2024). We showed that Ni isotope compositions correlate with PON concentrations as well as $\delta^{15}N_{PON}$ and are inversely correlated with nitrate concentrations (Figure 8). This suggests that enhanced primary productivity at stations with high $\delta^{60}$Ni in surface waters had led to the accumulation of a large PON pool by strongly consuming nitrate. Because microorganisms may face competition for nitrogen acquisition, microbial productivity, particularly bacterial, may thus rely on alternative nitrogen sources, such as urea. This has been observed in Arctic summer blooms, where metatranscriptomics abundance peaks of urease have been detected by multiple studies in the summer blooms (Royo-Llonch et al., 2021; Laso-Pérez et al., 2025). Interestingly, the genomes that dominated the transcription profiles of urease were Alphaproteobacteria and Gammaproteobacteria, matching the

annotations of gene clusters directly linked to $\delta^{60}$Ni (in yellow in Figure 4), which are all annotated as urease enzymes carried by proteobacterial families such as Rhodobacteraceae and Roseobacteraceae. Furthermore, Rhodobacteraceae strains living in interaction with diatoms have been shown to use urease as a nitrogen source in a culture-based study (Zecher et al., 2020). There are also multiple pieces of evidence for the use of urea as a nitrogen source by heterotrophic bacteria in the Arctic winter (Fouilland et al., 2007), and it has been proposed that sea ice could be a source of urea, which could explain the high abundance of urease at Mertz polynya (Conover et al., 1999). This specific nitrogen biogeochemistry in polynya waters could also explain the surprising heavy Ni isotope composition at the surface of station 11 compared to other high-latitude stations. In the high latitudes, microbial growth is typically supported by high nitrate concentrations from upwelling or nitrification processes (i.e., oxidation of ammonium to nitrite and nitrate). For example, in waters from the Kerguelen Plateau, nitrification has been shown to supply up to 50% of the total nitrate uptake in the mixed layer, thereby preventing any nitrogen limitation (Cavagna et al., 2015; Dehairs et al., 2015; Fripiat et al., 2015). Interestingly, no Ni isotope fractionation was observed in these same Kerguelen samples (Wang et al., 2019).

The shift in nitrogen biogeochemistry, together with the observed co-variations between $\delta^{60}$Ni and metagenomics data, may suggest that the Ni isotope fractionation reflects the removal of light Ni by the urease and/or Ni-SOD enzymes, independently of the taxa involved. Considering these results, the biogeochemical Ni divide between low and high latitudes may be driven by Ni metabolic requirements across contrasting regions facing limited nitrogen resource. Further work is required to explore this hypothesis. Measurements of urea concentration alongside Ni and nitrogen concentrations and isotopes in both dissolved and particulate phases would be beneficial. Extending these analyses to tropical/subtropical waters where cyanobacteria dominate the phytoplankton communities would also be needed, as the dominance of the urease and Ni-SOD enzymes over the NiFe hydrogenase enzyme in the present study may perhaps be explained by the weaker role of this latter enzyme in regions where cyanobacteria and diazotrophs are minor communities. Finally, repeated measurements of $\delta^{60}$Ni and meta-omics data from time-series would help to resolve any potential discrepancies in temporal scales between the datasets. Specifically, while meta-omics captures the instantaneous composition and functional state of microbial communities at the time of sampling, isotope signatures may integrate biological processes over longer periods, spanning days to months.

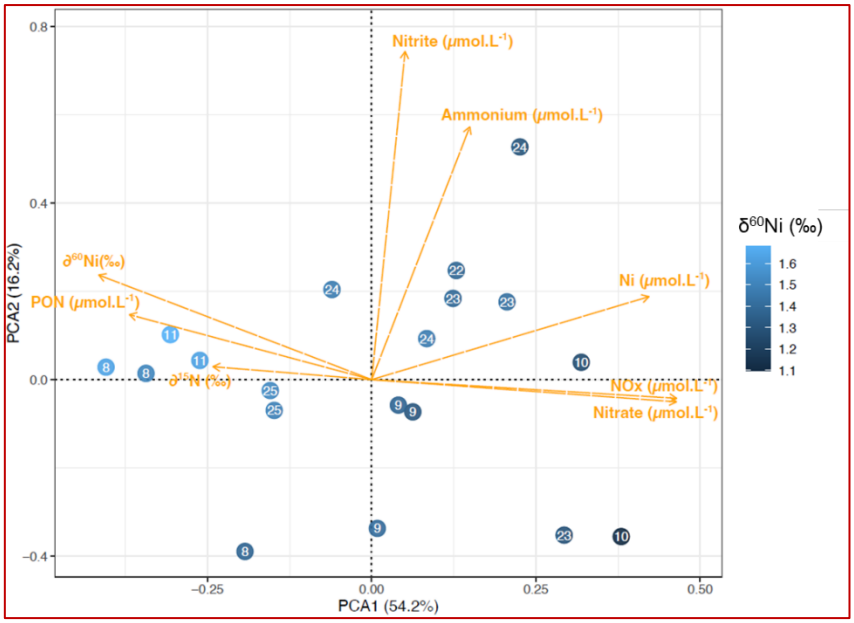


Figure 8: Principal Component Analysis (PCA) on the Ni concentrations and isotopes ($\delta^{60}$Ni) together with inorganic nitrogen concentrations (nitrate, nitrite, ammonium, NOx), particulate organic nitrogen (PON) concentrations and nitrogen isotope composition from PON ($\delta^{15}$N).

- *First paragraph introduction. Focusing on Ni-SOD which present in Cyanobacteria and Ostreococcus both not found in significant abundance in Southern Ocean waters.*

  Please note that our focus, at the start of the introduction, is not directly on the Southern Ocean, but instead we aim to provide an overview of the current state of knowledge regarding Ni. We also state in this paragraph that Ni-SOD is found in heterotrophic bacteria. Our results further show that the enzyme is both abundant and diverse at Southern Ocean scale (lines 439-442).

- *67 – Define biogeochemical divide.*

  The biogeochemical divide is a term used in the literature to describe the latitudinal dichotomy observed for Ni concentrations and $\delta^{60}$Ni in the global ocean: the lowest concentrations and heaviest Ni isotope compositions are observed in surface low latitudes, unlike in high latitudes. Hypotheses have been proposed to explain these heavy Ni isotope compositions in surface low-latitude waters. Please see our updated text below.

  Lines 63-69: Nevertheless, a biogeochemical divide in Ni vertical distributions has been reported between high and low-latitude oceanic regions, with surface depletion only measured at low latitudes (Sclater et al., 1976; Bruland, 1980; Middag et al., 2020). This biogeochemical divide also applies to Ni isotopes, with a significant fractionation in Ni isotopes in the surface only observed at low-latitude stations (Archer et al., 2020; Bian et al., 2024; Cameron and Vance, 2014; Lemaitre et al., 2022; Takano et al., 2017; Wang et al., 2019; Yang et al., 2021, 2020). This isotope fractionation demonstrates that one or more specific processes affect Ni biogeochemistry in low-latitude surface waters.

- *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.*

  Our goal here is to depict the potential mechanistic explanations behind the biogeochemical divide, at global scale and not only focusing on the Southern Ocean. We think that this general description is needed to properly present the motivations behind our selection of enzymes. However, we agree that discussing diazotrophs here could be misleading as the study focused on the Southern Ocean. We now have added a sentence to highlight the specificity of our study area in regards of the current Ni hypothesis.

  Lines 109-111: The Southern Ocean being a high-latitude environment mainly composed of nutrient-rich waters, we would expect to find heavy Ni isotope compositions only in surface of the lowest latitudes, as based on the hypotheses presented above (Archer et al., 2020; Lemaitre et al., 2022; Bian et al., 2024; Chiu et al., 2026).

- *80-92: Very long section on metagenomics which could be partly shortened or moved to methods. Depending if Biogeosciences 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.*

  We understand the referee's point of view, and agree that this paragraph was too long. We were confronted multiple times during the writing and review process to questions regarding the nature of meta-omics data, and feel like it is important that a geochemist with no experience in omics is able to get a good understanding of what we did and why. Still, we modified the paragraph in accordance with the referee's remarks.

  Lines 89-97: In this context, omics data appear as a promising tool to better understand the role of microbial communities and their activity in driving biogeochemical cycles (Sunagawa et al., 2015; de Vargas et al., 2015; Guidi et al., 2016; White et al., 2016). Sequenced metagenomics samples, called metagenomes, are composed of short DNA fragments (called reads) that can be assembled into longer sequences (called contigs) to recover a large fraction of the genes present in the original sampled population. The same DNA sequences can also be taxonomically and functionally annotated through comparisons with reference databases, helping to decipher the genetic potential of natural populations present in the environment (e.g., Vernette et al. 2022). Metagenomics thus provide critical insights into microbial diversity and metabolic and functional potential, thereby serving as a powerful approach for investigating the impact of bio-mediated processes on nutrient distributions in the ocean (Levine and Leles, 2021).

- *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

Please note that the data for Ni and metagenomics that we used were collected from the same stations, reducing to a minimum any potential scales issues. This important detail has been added in different sections.

Lines 107-109: Here, we present Ni concentrations, $\delta^{60}$Ni and metagenomics, all determined from the same stations and depths, in the upper 1000 m of the Atlantic sector of the Southern Ocean and along a transect between Tasmania and Antarctica as part of the Antarctic Circumnavigation Expedition (ACE).

Lines 118-121: During the ACE cruise (December 2016-March 2017, R/V Akademik Tryoshnikov), seawater samples were collected from the same stations for both Ni concentrations and isotopes (10-12 depths in the upper 1000 m) and for metagenomics (at 5, 15, 150 and 1000 m, complemented by other depths when possible). Sampling was undertaken at 8 stations from Leg 2 (stations 8-11) and Leg 3 (stations 22-25), in the Atlantic and Pacific sectors of the Southern Ocean respectively (Figure 1).

Caption of Figure 1: Location of the sampling stations for Ni concentrations, Ni isotopes and metagenomics during the ACE cruise, superimposed on a map of total surface chlorophyll-a concentrations from underway seawater samples (all small grey circles Antoine et al., 2020).

Metagenomics gives the immediate microbial composition at the time of sampling, while isotope compositions are more likely to reflect a timescale of days or weeks of biological activity. We now discuss these scale differences across our different data in the discussion.

Lines 512-515: Finally, repeated measurements of $\delta^{60}$Ni and meta-omics data from time-series would help to resolve any potential discrepancies in temporal scales between the datasets. Specifically, while meta-omics captures the instantaneous composition and functional state of microbial communities at the time of sampling, isotope signatures may integrate biological processes over longer periods, spanning days to months.

- *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.*

  This was modified accordingly.

  Lines 107-115: Here, we present Ni concentrations, $\delta^{60}$Ni and metagenomics, all determined from the same stations and depths, in the upper 1000 m of the Atlantic sector of the Southern Ocean and along a transect between Tasmania and Antarctica as part of the Antarctic Circumnavigation Expedition (ACE). The Southern Ocean being a high-latitude environment mainly composed of nutrient-rich waters, we would expect to find heavy Ni isotope compositions only in surface of the lowest latitudes, as based on the hypotheses presented

above (Archer et al., 2020; Lemaitre et al., 2022; Bian et al., 2024; Chiu et al., 2026). We provide new data complementing the still limited Ni isotope database in the ocean and aim to provide a clearer picture of where the Ni isotope fractionation occurs between low and high latitudes. Moreover, the high-resolution of the shallow depth profiles enables us to advance our understanding of surface processes driving Ni cycling and therefore to investigate the hypothesis that specific enzymes and microbial communities may play a role in the oceanic Ni biogeochemical divide.

- *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?*

  We apologize for not making our description of the data clearer. The metagenomics samples from the ACE campaign were collected at 5m, 15m, 150m and 1000m depth at each meta-omics station, and completed by other depths when possible. Depth profiles for Ni measurements were achieved at a larger depth resolution. In this study we used samples for which we had both metagenomics and Ni measurements taken from the same station at the same exact depth. The majority of these samples were taken at 15m (14 samples) or 150m (15 samples). The rest (19 samples) were taken at 30m (6 samples), 40m (2 samples), 50m (3 samples), 70m (2 samples), 80m (2 samples), 250m (1 sample) and 1000m (3 samples). This information was added to the methods.

  Lines 118-121: During the ACE cruise (December 2016-March 2017, R/V Akademik Tryoshnikov), seawater samples were collected from the same stations for both Ni concentrations and isotopes (10-12 depths in the upper 1000 m) and for metagenomics (at 5, 15, 150 and 1000 m, complemented by other depths when possible). Sampling was undertaken at 8 stations from Leg 2 (stations 8-11) and Leg 3 (stations 22-25), in the Atlantic and Pacific sectors of the Southern Ocean respectively (Figure 1).

- *& 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?*

  Please see our precedent answer. Note that we also modified this paragraph to focus on the metagenomics from this specific study. We thank the reviewer for noticing our mistake in the caption of Figure 1: we added that the symbols on the map represent the location of the sampling stations for Ni concentrations, Ni isotopes *and* metagenomics during the ACE cruise.

- *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.*

  We switched to past tense. We understand the referee's point, but we want this manuscript to be accessible by most geochemists and biologists. We therefore decided to keep this sentence to describe our methods as clearly as possible for non-specialists of omics.

- *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.*

  We thank the referee for their suggestion. We did not check Expazy. However, we did use FeGenie to investigate Fe-related enzymes in the global ACE dataset, but we could not see a good reason to use these annotations in this study focusing on Ni. These FeGenie annotations will hopefully be part of a future publication using the full span of omics samples available in the ACE data. What we did investigate and present in a previous version of this study is a larger set of genes related to Ni. We searched for all annotations related to Ni in the eggNOG annotations through specific word searches (e.g. "nickel", "Ni", …), which returned a set of genes vastly dominated by nickel transporting enzymes, mainly from the nik operon (e.g., nikM). Correlation between Ni transporters abundance and $\delta^{60}$Ni was similar to the one between Ni-SOD and $\delta^{60}$Ni in the small size fraction, and no correlation was found regarding transporters in the large size fraction (which is not really surprising as these transporters were mainly prokaryotic). These results were thus not bringing much to the main messages of this study, adding significant amounts of text in the methods and results section for a very limited impact. In addition, pooling together all transporters is less justified than working at single enzyme level, and the high diversity in their EggNOG annotations made it hard to produce satisfying visualizations and statistics. For these reasons we decided to remove them to focus only on the enzymes containing Ni, that had already been suggested as the potential cause of Ni fractionation in the literature.

- *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.*

  We understand the reviewer's concern, as this choice may be counter-intuitive in the Southern Ocean. However, we have decided to investigate all Ni-containing enzymes commonly found in marine plankton, including those impacting diazotrophs because one hypothesis about the Ni isotope fractionation was its link to $N_2$ fixation in the North Atlantic (Lemaitre et al., 2022). We have added a sentence describing this hypothesis in the introduction.

  Lines 79-80: In fact, the Ni isotope fractionation was found to correlate with nitrogen fixation in the North Atlantic (Lemaitre et al., 2022).

  Moreover, we would like to point out that both Ni-SOD and Urease are highly abundant and diverse in the ACE data. Our new analysis (see section 4.3 and Figure 8) showing the relationship between N-related variables and Ni measurements clearly highlights the interplay between the Ni and N cycles, even in the Southern Ocean where nitrogen fixation is mostly absent. We hope it will help to better convince the referee of the relevance of our approach.

- *230: Have you tried matching against https://academic.oup.com/ismej/arHcle/15/10/2933/7474411*

*https://www.nature.com/arHcles/ismej201231*
*https://pubmed.ncbi.nlm.nih.gov/23126454/*
*https://pubmed.ncbi.nlm.nih.gov/22806143/*

We thank the referee for taking the time to point towards these studies. We are not sure to understand what they mean by "matching". We assumed that they implied we could map our metagenomics data on existing collections of genomes from the Southern Ocean. In the sentence indicated by the referee (line 230) we were specifically talking about using genome-resolved approaches for better deciphering the functional diversity of Eukaryotes, which is something we have not done. Building MAGs of Eukaryotes and properly annotating their genes is far more complex than doing it for Prokaryotes, and none of the studies listed in the referee's comment is focused on Eukaryotes, so we believe this might have been unclear to the referee. We modified the sentence to make it clearer (Line 548: "to better integrate eukaryotes" was changed to "specifically targeting eukaryotes").
It would however be possible to do it using prokaryotic MAGs, to tackle a different question. This is actually something we have done using our own collection of prokaryotic ACE MAGs (~1500 MAGs built from ACE data, unpublished). The results were relatively inconclusive, as very distinct MAG communities were found at stations 11 and 8, telling basically the same story as the gene-level analysis we present here. Since the MAG collection remains unpublished as of now, we did not integrate the results in our manuscript.

- *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.*

  Please refer to our answer concerning lines 205-215 for the point concerning Nik genes. We now added the amount of Ni in each of the three enzymes : ~1 g-atom of Ni per mol of subunit in Urease (Christians & Kaltwasser, 1986), ~0.74 g-atom of Ni per mol of subunit in Ni-SOD (Youn et al., 1996) and ~1 g-atom of Ni per mol of enzyme for the NiFe hydrogenase (Cammack et al., 1994). This information has been added to the introduction. Regarding hydrogenase, it is already included in our study (Ni-Fe hydrogenase), while Ni-containing CO-dehydrogenase was deemed irrelevant in our case at it is an anaerobic enzyme and we are focused on surface waters outside of oxygen minimum zones.

  Lines 46-50: Among these different enzymatic roles, Ni is required as the metallocenter in 'urease' enzymes that recycles urea back to bioavailable ammonia (~1 g-atom of Ni per mol of subunit; Christians and Kaltwasser, 1986), in 'NiFe hydrogenase' enzymes that oxidises dihydrogen produced during nitrogen fixation (~1 g-atom of Ni per mol of enzyme; Cammack et al., 1994), and in 'Ni superoxide dismutase' (Ni-SOD) enzymes that degrades superoxide

radicals and other reactive oxygen species generated through photosynthesis and respiration (~0.74 g-atom of Ni per mol of subunit; Youn et al., 1996; Ragsdale, 2009).

To our knowledge the studies providing Ni quotas only focus on phytoplankton species: diatoms, flagellates, picophytoplankton in different oceanic regions (Twining et al., 2004, 2009, 2011, 2012, 2013, 2015). We could not find a similar study focusing on heterotrophic bacteria. Overall, the number of studies focusing on Ni as a trace element in planktonic ecosystems is extremely scarce compared to the vast literature focusing on iron. We find that this is one of the reasons making our exploratory analysis relevant.
Concerning the taxonomy of AGCs, we decided to avoid a long and wordy description of the taxonomy of the multiple AGCs in the main text, instead providing a table with annotations for the key clusters (Table 1). This table is cited in sections 3.2.2 and 4.2. We feel like it is more relevant to discuss taxonomy and abundance at the scale of each AGC rather than providing enzyme-level taxonomic diversity. There could for example be many AGC annotated as urease coming from a variety of low abundance taxa, impacting the apparent taxonomic diversity of urease without having real ecological relevance. Working at AGC level allows us to focus only on the taxonomy of the most ecologically relevant clusters at our stations of interest.

- *357: explain how this is consistent with the biogeochemical divide*

  We modified the sentence to make it clearer. Note that we also defined the biogeochemical divide in the introduction (see response to your comment on line 67).

  Lines 374-376: Overall, the new ACE dataset follows established oceanic Ni-$\delta^{60}$Ni systematics and is consistent with the biogeochemical divide typically reported between low and high latitudes: lower concentrations associated with heavier isotope composition in surface low-latitude waters.

- *430-445: but how do you link this to existing literature and information to these heterotrophic bacteria?*

  We now discuss these findings in relation with the existing literature:

  Lines 445-454: 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 (Tan et al., 2016). Porticoccaceae were identified as the dominant contributors to Fe and Mn transporters in waters above the Kerguelen Plateau, while Nitrincolaceae, which were not associated with any of our gene clusters of interest, accounted for most of the signal in Ni transporters, followed by Rhodobacteraceae (Kong et al., 2024). The same study identified an Ni-SOD peak during the post-bloom period, without discussing its taxonomic origin (Kong et al., 2024). The urease enzyme is mostly carried by alphaproteobacterial families such as Rhodobacteraceae and Roseobacteraceae (Tables 1 and S2), matching previous observations from the Arctic (Royo-Llonch et al., 2021; Laso-Pérez et al., 2025).

- *451: Difficult to know without urea measurements*

  We agree with the referee, and we are only trying to draw a hypothesis here. However, we added sentences highlighting the need for urea measurements combined with Ni measurements to properly demonstrate this.

  Lines 508-509: Measurements of urea concentration alongside Ni and nitrogen concentrations and isotopes in both dissolved and particulate phases would be beneficial.

  Lines 546-549: This should include analyses of urea concentrations in parallel of Ni and nitrogen concentrations and isotopes, a more complete analysis of microorganisms' metabolisms through genome-resolved approaches specifically targeting eukaryotes, and the use of metatranscriptomics to examine the activity of Ni-containing enzymes in addition to their presence.

- *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?*

  Thank you for noticing this mistake of vocabulary. We meant to discuss the potential use of urease as an explanation for Ni fractionation but this, indeed, cannot be described as its activity with our data. We have modified this sentence to make it clearer.

  Lines 468-471: It is therefore possible that diatoms and *Rhodobacteraceae* have used urea as a nitrogen source, potentially impacting carbon and ammonium availability in a context of high primary productivity (see section 4.3). This potential uptake of urea at station 11 could have led to a greater Ni utilisation through the urease enzyme.

- *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.*

  We now discuss this aspect in more depth, notably presenting multiple findings of high urease concentrations and heterotrophic bacterial utilization in the Arctic Summer blooms: https://journals.asm.org/doi/full/10.1128/mbio.00749-25 Urea uptake and assimilation genes expressed in bacteria during Arctic bloom: "In mid-June, the Alphaproteobacteria_21 (comp. 98.3%, cont. 0.5%) MAG dominated the ureABC metatranscriptomic profiles"; "Urea can serve as a nitrogen, carbon, and energy source. In our data set, the transcription peak of ureABC in mid-June could suggest an intense usage of urea by Alphaproteobacteria and Gammaproteobacteria during the bloom. Arctic urea concentrations are usually below 1 µM, but tend to be higher in summer (16, 80–82), probably due to the release of the urea accumulated in the winter ice (70). In line with this fact, Royo-Llonch reported transcription of urease genes in summer samples for several Arctic MAGs (13), and summer urea uptake has been measured in different Arctic waters (16, 80, 82)."
  https://academic.oup.com/plankt/article/29/4/369/1667237?guestAccessKey= In Autumn samples from the Arctic heterotrophic bacteria are responsible of 5-42% of urea uptake

https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2020.533894/full use of urea as nitrogen source by a rhodobacteraceae strain interacting with Diatoms.

https://journals.asm.org/doi/full/10.1128/aem.01431-14 use of urea as main nitrogen source by bacteria and archaea in the Arctic winter (ammonium in the summer)

Lines 488-496: This has been observed in Arctic summer blooms, where metatranscriptomics abundance peaks of urease have been detected by multiple studies in the summer blooms (Royo-Llonch et al., 2021; Laso-Pérez et al., 2025). Interestingly, the genomes that dominated the transcription profiles of urease were Alphaproteobacteria and Gammaproteobacteria, matching the annotations of gene clusters directly linked to $\delta^{60}$Ni (in yellow in Figure 4), which are all annotated as urease enzymes carried by proteobacterial families such as *Rhodobacteraceae* and *Roseobacteraceae*. Furthermore, *Rhodobacteraceae* strains living in interaction with diatoms have been shown to use urease as a nitrogen source in a culture-based study (Zecher et al., 2020). There are also multiple pieces of evidence for the use of urea as a nitrogen source by heterotrophic bacteria in the Arctic winter (Fouilland et al., 2007), and it has been proposed that sea ice could be a source of urea, which could explain the high abundance of urease at Mertz polynya (Conover et al., 1999).