We thank Jill Sutton for her thoughtful and constructive comments. Her comments are listed below, and our responses are provided in blue.

Grasse et al. provide the first *in situ* estimate of δ³⁰Si fractionation for silicoflagellates, which is a new and important contribution to understanding Si dynamics in the modern and past marine environments. The inclusion of the 2017 KOSMOS mesocosm experiment adds novelty by offering a controlled environment to study Si isotope fractionation, a major limitation in field-based studies. While the manuscript is well written and informative, several sections would benefit from clearer articulation of the scientific objectives, improved structure, and a more detailed explanation of uncertainties. In addition, some broader implications were underdeveloped given that their short summary indicates that this information would be "providing a novel tool for understanding dSi utilization in the past. The paper could strengthened by more explicitly connecting the results to: (1) global δ³⁰Si budgets, (2) paleoceanographic reconstructions (3) potential biases in interpreting δ³⁰Si (water column and sediment). Below is a list of comments suggesting both major and minor revisions.

1) Global δ³⁰Si budget: A major uncertainty in evaluating the influence of silicoflagellates and their higher Si isotope fractionation factor comes from the fact that we lack reliable global estimates of silicoflagellates abundance in the modern ocean. Most authors cite Riedel (1959), who reported that silicoflagellates skeletons typically account for only 1-2% of the siliceous fraction in marine sediments. While this provides a useful sedimentary benchmark, it does not necessarily reflect their standing stock or production in the water column, as their abundances can be very patchy, seasonally variable, and regionally restricted. Without better observational data, it is challenging to determine whether their higher fractionation factors could meaningfully bias large-scale silicon isotope estimates. However, while we do not expect silicoflagellates to exert a major influence on the δ³⁰Si budget in the modern ocean, the situation may have been different in the geological past. Several studies indicate that silicoflagellates experienced periods of much higher abundance, particularly during the Late Cretaceous to early Paleogene. During these intervals their contribution to biogenic silica production may have been large enough to influence local or even basin-scale silicon-isotope signatures. While we can only hypothetical assumptions about their potential impact, dedicated studies would be required to properly evaluate this possibility.

Point 2) and 3) are discussed in detail below. The new information will be added to the discussion.

Major comments:

The introduction provides extensive background on the Peruvian Upwelling, Si cycling dynamics, plankton community dynamics and Si isotopic fractionation, but the primary research questions or hypotheses of the study should be more clearly presented. For example, the central scientific question is a little bit buried: "What is the Si isotope fractionation factor for silicoflagellates, and how does it influence δ³⁰Si in dynamic upwelling systems?". This should be explicitly stated towards the end of the Introduction.

We will add further information to the last paragraph of the introduction.

*"In 2017, we conducted a KOSMOS (Kiel Off-Shore mesocosms for Future Ocean Simulations) experiment off Lima (Peru) for 50 days to simulate upwelling with different nutrient stoichiometries*

*(N:Si and N:P). While upwelling of nutrient rich water in the Peruvian upwelling generally induce diatom blooms (e.g. Franz et al., 2012, Grasse et al., 2021), low N:Si ratios would lead to shifts in the phytoplankton communities. The unique setting of the experiment made it possible to study the evolution of $\delta^{30}Si_{dSi}$ and $\delta^{30}Si_{bSi}$ in a closed system without the influence of horizontal water mass mixing. After the addition of nutrient depleted deep water, we observed a shift from a diatom-dominated community (day1-10, phase I) to a (silico-)flagellate-dominated community (day 13 to 42, phase II). This shift provided novel insights into the silicon cycle in the Peruvian upwelling system and enabled, for the first time, the determination of a silicon isotope fractionation factor for silicoflagellates. Importantly, these data also allow, for the first time, an assessment of the potential influence of silicoflagellates on the dissolved silicon isotope composition in upwelling regions, and thus under which environmental conditions their contribution may exert a measurable effect on the marine Si isotope distribution.*

While diatom dynamics are thoroughly explained in the introduction, a discussion of (silico) flagellates is somewhat brief and lacks detail. Given that the study presents the first Si isotope fractionation factor for silicoflagellates, more ecological and physiological context (and references) would be useful. For example, lines 69-73 lack references and there appears to be an error in the paragraph. Some questions to address include: (1) How do silicoflagellate silica structures differ from diatom frustules? (2) What is known about their silica uptake pathways? The emphasis of what is unknown will help the reader understand the importance of the questions being addressed.

The sentence (former L69-73) was corrected as follows: "These shifts in phytoplankton composition strongly affect the marine silicon cycle, but also the carbon cycle. While carbon uptake rates in some silicoflagellate species (e.g. *Dictyocha perlaevis*) have been shown to be comparable to other phototrophic phytoplankton species (e.g. Taguchi & Laws 1985), there are still insufficient data on carbon uptake differences between different species of silicoflagellates or changes caused by the variability of environmental conditions (Closset et al. 2025)."

We also added further information to the introduction:

1)  How do silicoflagellate silica structures differ from diatom frustules?

    While diatoms have two overlapping valves, the silica skeleton of silicoflagellates is an external, intricate, and rigid framework of hollow, opaline silica tubes, forming a basket-like structure (Preisig 1994). For few species it was possible to show that they can have a double skeleton (sometimes interpreted as pre-division stages). The paired skeletons are not mirror images but share the same rotational orientation, forming dome-shaped halves of a more spherical structure, a pattern with important implications for skeletal function, phylogeny, and the generic distinction of living and fossil silicoflagellates (McCartney et al. 2014).

2)  What is known about their silica uptake pathways?

To our knowledge there are no studies on the silica uptake pathways in silicoflagellates

Also, I found that the presentation of the KOSMOS mesocosm experiment in the introduction lacked some detail. A clearer statement of the study targets (i.e. specific processes) and why these findings mater for broader oceanographic or paleoceanographic applications should be highlighted.

The KOSMOS study investigated the effects of upwelling of deep waters with varying N:P and N:Si ratios, and how these ratios influence the composition of phytoplankton and zooplankton. However,

the induced upwelling exhibited lower N:P and N:Si ratios than anticipated, leading to unexpected conditions such as a silicoflagellate bloom.

Silicoflagellates are often overlooked due to their lower abundance compared to diatoms. This lower abundance in surface waters is reflected in the sediments below: While they are present in several of the shorter sediment cores from the Peruvian upwelling (personal communication Kristin Doering), they likely play a dominant role for biogeochemical conditions under which diatoms are absent. Their occurrence following diatom blooms may also help constrain the role of non-diatom silicifiers in the silicon cycle, and knowledge of their fractionation factor can potentially improve estimates of $\delta^{30}Si$ signatures of dissolved silica under nutrient-limiting conditions, allowing an assessment of whether DSi was fully consumed or whether nitrate limitation prevailed.

We will add further clarification to the last part of the discussion.

While nutrient supply mechanisms (vertical advection, mixing, seasonal variability) are described accurately, the manuscript somewhat underplays mesoscale variability, differences in water mass sources, and timescale interactions (physical vs. biological). All of these can strongly shape local $\delta^{30}Si$ signatures and community composition. Briefly incorporating these factors would produce a more comprehensive discussion of why field-based $\delta^{30}Si$ measurements are difficult to interpret.

We agree and will incorporate a sentence that highlights the complexity of temporal and spatial evolution of hydrographic conditions and changes in physical and biological interactions and their importance for the resulting dissolved and particulate $\delta^{30}Si$ signal. The following sentence was added to the discussion L516:

*"Overall, the interpretation of silicon isotopes in field studies is complicated, not only due advection of differencent water masses (horizontally and vertically), but also due to processes on different time scales. While seawater can integrate a signal of Si utilization as well as dissolution on rather short time scales, the particles reflect the evolution of the signal over longer time scales."*

The critique of Rayleigh and steady-state models is important, but the manuscript does not fully explain how these limitations influence isotopic interpretations in practice. The mesocosms are semi-closed, but episodic mixing (bottom layer intrusion, biomass movement) introduces potential non-Rayleigh effects. This should be more thoroughly addressed. Specifically: (1) Do oversimplified $\delta^{30}Si$ assumptions bias estimates of Si utilization? (2) Are there specific examples from past studies where these biases have been demonstrated and/or discussed?

Vertical mixing in the mesocosms was minimized by adding saltwater to the bottom layers on days 13 and 33 (see Bach et al., 2020). Mixing between surface waters and the deep-water additions mainly occurred prior to the additions and around day 30; therefore, the days for which the Rayleigh fractionation model was applied to estimate the silicoflagellate fractionation factor experienced only minimal vertical mixing. Although the Rayleigh model likely provides the best estimate under these conditions, we now clarify in the text, that the surface layer was not a completely closed system given that minor mixing events, dissolution, and biomass sinking may have influenced surface water $\delta^{30}Si$ values. Previous field studies in the Peruvian upwelling have shown that the Rayleigh-type fractionation model often underestimates the fractionation factor of diatoms (Ehlert et al. 2013; Grasse et al. 2021), due to upwelling and effects of dissolution.

As Kosmos 1 contains a larger fraction of diatoms, which have growth rates different from silicoflagellates (see comments reviewer 1), we now only use Kosmos 2 and 7 for the calculation of the Si isotope fractionation factor of silicoflagellates.

While we are confident that the fractionation factor of silicoflagellates is significantly higher than that of the investigated diatom species, we will add additional context to the manuscript clarifying uncertainties and that further studies, particularly culture experiments, are needed to verify the silicoflagellate fractionation factor.

Minor comments:

For the silicoflagellate ε calculation, the assumption that silicoflagellates dominate uptake between days 13–17 is reasonable but requires more explicit demonstration (perhaps with size-fraction bSi?). Also, is there a possible role of dissolution (diatom or other organisms) in this zone? How would this influence the interpretation of the ε30Si?

We will include further information on the diatom species, size distribution as well as biovolume specifically for day 13 and 17.

Dissolution likely occurs throughout and would tend to lower the apparent fractionation factor for silicoflagellates. This effect is illustrated in Figure 6, where $\delta^{30}Si$ is plotted against ln(dSi), a relationship governed by the balance between silicate utilization and dissolution, with the slope reflecting the net fractionation. Fully constraining the magnitude and role of dissolution, particularly for silicoflagellates, requires further investigation, which is beyond the scope of this study.

We will add a paragraph to the Discussion section addressing potential processes that may influence the estimated silicon isotope fractionation factor of silicoflagellates.

Line 106 - "admixture from multiple sources"  Do you mean "a mixture.."?

Will be corrected

Lines 84-92 - Also, see Frings et al. 2024 for newer information.
.https://doi.org/10.1016/j.quascirev.2024.108966

We searched for additional information in Frings et al., 2024 and will add a sentence in L90:

*"Further experiments suggest that isotopic fractionation is primarily driven by early kinetic effects during rapid silica precipitation rather than biomolecule-specific processes (Cacarino et al., 2021)."*

Line 540 – perhaps cite Cotard et al. 2025 (https://doi.org/10.1002/lno.70243) for the lithogenic input ? They have some interesting evidence supporting that lithogenic input could affect the dSi composition. Is there any other evidence that could support this argument? For instance, rainfall, sediment load, turbidity, wind direction, proximity to dust sources?

We agree and will modify the sentence accordingly:

*"Such values could instead be explained by input of small lithogenic particles characterized by low $\delta^{30}Si_{dSi}$ (-1 to -3 ‰; Sutton et al., 2018). Even lower values (-0.33 ‰) in surface waters were observed above the Northern Kerguelen Plateau, clearly influenced by lithogenic input (Cotard et al., 2025)."*

As we described in the manuscript, the Coastal El Niño affected the region around the mesocosms through heavy rainfall and enhanced sediment load from the rivers (e.g. Rodríguez-Morata et al. 2019; Geilert et al. 2023). Unfortunately, the additional datasets provide no further insight into

potential lithogenic inputs. The trace-metal measurements (e.g., Fe, Cd, Zn) varied widely with no clear pattern, most likely caused by contamination within the mesocosms (pers. communication Mark Hopwood). Likewise, the PAR sensor data offer no evidence for enhanced particle loads or light attenuation that would indicate lithogenic material entering the system.

We know from personal communication that the wind pattern had shifted at the end of the experiment, which transported very small dust particles. We plotted the wind direction and speed for day 40 to 47 (beginning of Apil 2017) and the last days of the experiment (day 48 and 50) with ERA5 hourly data on single levels from 1940 to the present (https://cds.climate.copernicus.eu/datasets/reanalysis-era5-single-levels?tab=overview) Unfortunately, the resolution is too low to show the local wind situation (Fig. R1, Experiment location marked by grey box). Wind data from the Isla Lorenzo weather station near Lima were available only as monthly averages and therefore do not capture short-term wind variability (Fig. R2).
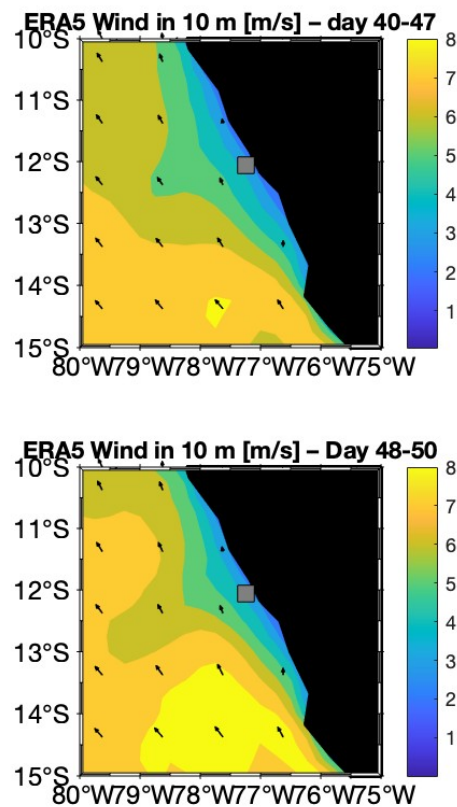


*Fig. R1: Wind direction (arrows) and speed (color) for the study area before small dust particles were transported inside the mesocosm (day 40-47) and thereafter (da 48 -50). The grey square indicates the location of the KOSMOS Experiment.*
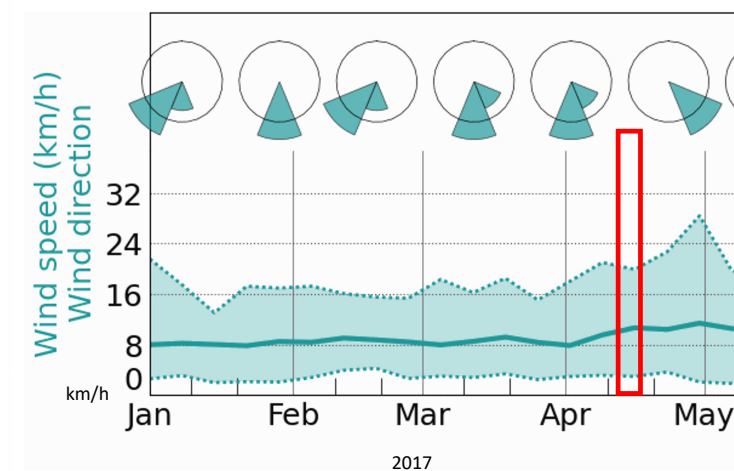
*Fig. R2: Wind direction and speed (m/s) data from a weather station on Isla San Lorenzo (Close to Lima) are only available monthly from January until April 2017 (Data source: Meteoblue). The last two sampling days of the experiment are marked by a red bar (14th and 16th of April).*

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