Reviewer 1

We thank the reviewer for their thoughtful and constructive comments. The reviewers' comments are listed below, and our responses are provided in blue.

Grasse et al investigated the Si processes regulated by diatoms and silicoflagellates by pelagic mesocosms in coastal Peru upwelling areas and stable Si isotopes. Overall, this is an interesting study and provide important knowledge of Si isotope fractionation of -3.63 ‰ during silicoflagellate production, implying a potential important application when investigating sedimentary records of biogenic Si. Prior to its publication, I have the following major and minor comments.

Major comments:

Regarding the calculation of Si isotope fractionation factor for silicoflagellates, the authors are missing an in-depth assessment of uncertainties. Given the data, diatoms and silicoflagellates are growing at different rates and exhibiting different abundance over the entire period. During the first 10 days, the production is exclusively dominated by diatoms, while thereafter, silicoflagellates take over within days. This indicates the growth rate of silicoflagellates is higher than diatoms and meanwhile consume more DSi relative to per unit cell of diatoms (this seems possible to be estimated given the experimental data), which in turn raise a question of what is a proper number for the initial d30Si value of DSi for them to grow. It is fine to use Eq 6 to estimate a mixed 30ε value, but this is probably only valid when assuming both diatoms and silicoflagellates keep their growth rate constant and the difference between these two growth rates remain the same. This means, even if they can share the same d30Siinit value derived from DW, the fraction of DSi, f, could be varied at each time point for diatoms and silicoflagellates. Subsequently, Eq 7 calculates the 30ε for silicoflagellates using relative abundances, but since their Si/cell differ much as shown in Fig 5b, should this be taken into account in the f in Eq 7?
Perhaps I make this question overcomplicated, but another thought is M2 and M7 is dominated by silicoflagellates by over 97% in certain days, why we need to bother the mixture with diatoms? It could be straightforward to use those data in those days to directly calculation 30ε for silicoflagellates.
I may miss some important information in this, but since this is the fundamental part and the main conclusion of the ms, such uncertainties should be clarified before publication.

We agree, that the simplified equation may not capture all the factors influencing the fractionation factor, such as growth rate or bSi content per cell. However, adding additional parameters to the equation would even induce more uncertainties, as growth rates as well as BSi content per cell vary not only between diatoms and silicoflagellates, but also between different diatom species.

We recalculated the fractionation factor for Kosmos 1 (a mixture containing diatoms and silicoflagellates) using biovolume data from Olenia et al. (2015) instead of cell counts for both groups on Day 17. It should be noted that phytoplankton species can vary greatly in cell size, as reflected in the biovolume, and not all cell counts refer to specific taxa (e.g. listed as pennates or only the genus), which leads to further uncertainties. Using biovolume instead of cell counts, the fractionation factor for M1 is lower (-2.7 ‰) compared to the previous estimate (-3.71 ‰) as the biovolume for diatoms only accounts for 9 % compared to 91 % for silicoflagellates (cell counts were: 37.74 % diatoms, 62.27 % silicoflagellates)

We agree with the reviewer that estimating the Si isotope fractionation factor in experiments with mixed diatom and silicoflagellate assemblages is more complex and potentially biased. We therefore restricted our analysis to fractionation factors from two independent experiments (Kosmos 2 and Kosmos 7), where silicoflagellates comprised up to 99% of the biovolume and were thus consistent

with the corresponding cell counts (97–98%). The new mean fractionation factor for silicoflagellates is −3.54 ‰, which is, however, within the uncertainty range of the previously estimated value of −3.63 ‰.

While we are confident that the fractionation factor for 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 Si isotope fractionation factor for silicoflagellates.

The table and text will be adjusted accordingly. Equation 7 will be removed from the manuscript. The revised Table 2 is presented below. The mean biovolume data will be added to the main text.

Kosmos	Day 13	Day 13	Day 17	Day 17	*f*	$^{30}\varepsilon$(Silicos)
	dSi (initial)	δ^{30}Si (dSi, initial)	dSi (final)	δ^{30}Si (bSi)		‰
M2	8.90	2.93	5.91	0.12	0.66	-3.47
M7	5.97	3.40	3.56	0.65	0.60	-3.60
				Average		**-3.54**
				2 s.d.		**0.18**
				(2 s.d Monte Carlo)		**0.40**

Other suggestions:

For clarity, it is better to say Si isotope fractionation factor in the text, or at least isotope fractionation factor, instead of “fractionation factor”.

We agree and will adjust this accordingly throughout the text.

Line 47 the superscript “-” should be removed.

Will be removed

Line 70 “dinoflagellates” should start with the captial letters.

We will correct the sentence and add further information.

“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 is still insufficient data on their carbon uptake to allow comparison of different species of silicoflagellates or changes in environmental conditions (Closset et al. 2025).”

Line 184 “at” should be removed.

Accepted

Line 173-174 vs Line 207-209 Why BSi was digested at different NaOH concentration and temperature? Are there any specific reasons? And for BSi contents and its Si isotope measurement, how the authors assess the contribution from non-biogenic Si particles?

There are several protocols for determination of bSi concentrations with slightly different NaOH concentrations, temperatures. The bSi concentration during the mesocosm experiment was determined by the Mesocosm Team, which applied a method involving 0.1 NaOH at 85°C (see section 2.2). We used a protocol adapted from Mark Brzezinski's laboratory (UC Santa Barbara) involving 0.2 N NaOH in a 90 °C water bath to prepare samples for Si isotope measurements (please note that the text stated 95 °C, which will be corrected). In a second leach step, the filter was treated with 0.5 ml of 2.5 M HF for 48 hours to dissolve lithogenic material. To determine the optimal digestion times for silicon isotope measurements, we used test filters from different days with varying diatom and silicoflagellate abundances. Tests were conducted for a maximum of 150 minutes. A steep increase in the bSi content signal was observed during the first 80 minutes, given that bSi dissolves faster than lithogenic material. No significant increase in bSi was detectable thereafter (less than 5%), except for 2 samples from Day 1, which contain more lithogenic Si (up to 18%). This will be noted in the manuscript, and the corresponding data points will be highlighted in Figure 4.

Although Ragueneau and Tréguer (1994) pointed out that up to 15% of lithogenic silicate (LSi) can dissolve during sodium hydroxide digestion, our own measurements provide evidence that LSi dissolution in our samples, especially the samples used to determine the fractionation factor, was much lower. Assuming that the samples contained up to 15% LSi, the reported δ^{30}Si values may be underestimated by 0.2‰, assuming a mean isotope signature of −1.07‰ for clay minerals. Lithogenic primary minerals are heavier at −0.2‰ (Sutton et al., 2018), resulting in an offset of 0.02‰. Both values are within the analytical error margin. We did not measure Al/Si ratios in the bSi samples as these may have been heavily biased within the mesocosms, which are not trace metal-free. Secondly, as we demonstrated in Grasse et al. (2021), the correction method with Al has its limitations, as the Al/Si ratios in bSi samples depend on external factors and the conditions of the diatom cells (living versus dead) and do not exclusively indicate contamination with lithogenic material.

However, we would like to point out, that bSi samples, which were used to determine the fractionation factor (day 17) only contained negligible amount of LSi (3%).

We will add this information to the manuscript.

Line 315 and some relevant text in the result section. What is the application of DIP in this study? Are they just used to show DIP is not a limiting nutrient for primary producivity?

The information on dissolved inorganic phosphate (DIP) was added to the results section, as it is a relevant parameter during the mesocosm experiments. We therefore would like to keep Figure 2d and add further content to the manuscript.

Line 600-605 I agree that we should be more careful when using sedimentary bSi to reconstruct dSi utilization. But this is also dependent on the relative abundance of each bSi species in sediment records of the studied area and the purification of these species for isotope analysis.

We agree with the reviewer and will add additional information to the last section of the main discussion part:

"δ^{30}Si data obtained from siliceous phytoplankton (e.g., diatoms, radiolaria, and sponge spicules) in sediment cores have been used to gain insight into the mechanisms controlling the Si cycle of the past (e.g., Doering et al., 2016a; Doering et al., 2016b; 2019; 2021; Hendry & Robinson, 2012). However, reconstructing past dSi concentrations and utilization requires knowledge of species abundances in the sediment, in addition to careful purification of the samples used for Si isotope analysis. Further insights can be obtained from the fractionation factor associated with dSi uptake. Despite several studies that

investigated silicoflagellate abundances in the past (e.g., Bukry, 1981; Amigo, 1999; McCartney, 2013;), no studies have been conducted so far on δ^{30}Si signatures of silicoflagellates preserved in sediments. The obtained fractionation factor for silicoflagellates, therefore, provides the basis for the establishment of a new paleo proxy for the reconstruction of the Si cycle of the past."