Dear anonymous referee1

We will send responses to referee's comments of "Substantial nutrient consumption in the dark subsurface layer during a diatom bloom: the case study on Funka Bay, Hokkaido, Japan" (revised title) by Umezawa et al. The referee's comments were very helpful, and we have revised the manuscript taking all comments. Corrections in the revised manuscript (and the responses to referee's comments) are highlighted.

Best regards,

Corresponding author: Atsushi Ooki

Major points of revision

* We added discussions about diffusive transport of nitrate and subduction as possible reasons for nutrient reduction in the dark subsurface layer.
* We additionally carried out the dark incubation experiment using Thalassiosira culture with n = 4 samples.
*We additionally carried out the dark incubation experiment using natural seawater collected on 8 March 2022 before the peak of diatom bloom 2022.
*We revised the chapter structure.

Revised chapter structure

Abstract
1. Introduction
2. Material and methods
   2.1 Shipboard observations
   2.2 Analytical procedures
   2.3 Incubation experiments to test for nutrient consumption by diatoms in darkness
   2.4 Water mass types and mixed-layer and euphotic-zone depths
   2.5 Spatial distributions of temperature, salinity, and density at the sea surface
3. Results
   3.1 Hydrographic features
   3.2 Biogeochemical parameters
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4. Discussions
   4.1 Nitrate consumption in the dark layer between 15 February and 4 March
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      4.2.3 Diffusive transport between the surface and the subsurface layers
      4.2.4 Subduction of surface water into the subsurface layer
   4.3 The influence of nutrient consumption by diatoms in the dark subsurface layer
5. Conclusion

Response to referee 1

Reviewer's comment 1

1) Effects of the physical processes.

The authors denied the vertical mixing and horizontal mixing are the main reasons to decline the nutrient concentration at 30–50 m depth of this area. However, the water density on 15th March is lighter than that of 4th March. The authors did not show the horizontal distributions of density and nutrient concentration. So the subduction processes cannot be denied. When the authors have much data, the nutrient(nitrate)-density plot would help discussion on it. I am concerned about the possibility that the low-nutrient water formed in the euphotic layer in the other area subducted at the observation station. For example, such a phenomenon occurs in an anticyclonic eddy.

Respond to the comment 1

We added a discussion about the subduction with new figures as follows:

Line 163-171

2.5 Spatial distributions of temperature, salinity, and density at the sea surface
Spatial distributions of temperature, salinity, and density at the sea surface (1 m) were obtained from an ocean reanalysis product provided by Meteorological Research Institute in Japan. This is produced with an operational system for monitoring and forecasting the status of coastal and open-ocean waters around Japan (the JPN system; Hirose et al., 2020). The JPN system includes a double-nested ocean model, the core of which is a Japanese coastal model with a horizontal resolution of 2 km. Three sub-models are interconnected using a nesting technique: a global model (horizontal resolution ~100km), a North Pacific model (horizontal resolution ~10km), and Japanese coastal model (horizontal resolution ~2km). A four-dimensional variational method is applied to the North Pacific model as the assimilation scheme. The process of tides and river runoff are taken into consideration in this JPN system. See the technical report for more detail (Hirose et al. 2020).

Line 424 - 440

4.2.4 Subduction of surface water into the subsurface layer

Fourth, we discuss a possibility if subduction of surface water caused the decrease in nutrient concentrations at the subsurface layer (30 – 50 m) of the observation station 30. At the medium depth (40 m) of the subsurface layer, temperature, salinity, and density were 3.5 – 3.6 °C, 33.64, and 26.7σ, respectively, on 4 and 15 March (Fig. 2). Suppose surface water in certain area of the bay subducted and it reached 40-m depth at the observation station on 15 March, the subducted water should have the same temperature, salinity, and density as it had been at the surface. The average current speed at 40-m depth between these dates was 3.3 cm s^{-1} (unpublish data), which was obtained from acoustic doppler current profiler (ADCP) set on the sea floor at the station. The middle layer water at the station could have reached from anywhere of the bay within 11 days. We obtained the spatial distributions of temperature, salinity, and density at the sea surface (1 m) on 4 March using the ocean reanalysis product provided by Meteorological Research Institute in Japan (Fig. 9a-c).
From these spatial distributions, there was not any area that satisfied required temperature (3.5 – 3.6 °C), salinity (33.64), and density (26.7σ) to form subduction water at 40-m depth of the observation station, see an enlarged map of Fig. 9c. We considered that the subsurface layer water at the station was not

associated with subduction. Thus, we excluded a possibility of subduction as a reason for the nutrient decline.

Reviewer's comment 2

The other possible process is the nutrient diffusion process. I don't know the diffusion of this area, > several tens μmol N m−2 d−1 usually occurs in the ocean. The nutrient diffusion occurs with physical disturbance, but diapycnal nutrient flux must be considered. The observations were snapshots, and the authors may not observe the diffusion processes, but the authors must show the nutrient flux at 50 m and 30 m depths are balanced on 4th and 15th March based on the slope of the nitracline and pycnocline, and this process is not the major nutrient decline process.

Respond to the comment 2

According to the referee's comment, we calculated the diffusive transport of nutrients. We added a discussion about the diffusive transport of nitrate as follows:

Line 410 - 423

4.2.3 Diffusive transport between the surface and the subsurface layers

Third, we discuss an effect of diffusive transport of NO_3^- on concentration decrease at the subsurface layer (30 – 50 m) on 15 March. There is not any previous study to have measured diffusive coefficients (Kρ) in Funka Bay. We referred a range of Kρ (= 10^{-6} – 10^{-5} m^2 s^{-1}) measured just below the mixed layer (~ 30 m) at the western subarctic Pacific in summer (Dobashi et al. 2021). Concentration gradients of NO_3^- were –0.000221 μmol m^{-4} (= $\Delta NO_3^-{}_{20m–30m}$ / 10 m), –0.000141 μmol m^{-4} (= $\Delta NO_3^-{}_{30m–40m}$ / 10 m), –0.000115 μmol m^{-4} (= $\Delta NO_3^-{}_{40m–50m}$ / 10 m), and –0.0000135 μmol m^{-4} (= $\Delta NO_3^-{}_{50m–60m}$ / 10 m). The range of diffusive transport of NO_3^- were calculated to be 0.00022 – 0.0022 μmol m s^{-2} between 20 m and 30 m, which could result in concentration change of 0.021 ~ 0.21 μmol L^{-1} at 30 m for 11 days. Concentration changes between 30 m and 40 m and between 40 m and 50 m were calculated to be 0.013 ~ 0.13 μmol L^{-1} and 0.011 ~ 0.11 μmol L^{-1}, respectively. The sum of concentration changes at 30 m, which include transports from 20 m layer and 40 m layer, ranges from –0.20 μmol L^{-1} (= –0.21 + 0.013) to +0.11 μmol L^{-1} (= –0.021 + 0.13). Ranges of the sum of concentration changes at 40 m and 50 m were –0.12 ~ +0.096 μmol L^{-1} and –0.11 ~ –0.024 μmol L^{-1}, respectively. The observed decreases were of 1.6 μmol L^{-1} at 30 m, 2.0 μmol L^{-1} at 40 m, and 2.4 μmol L^{-1} at 50 m between these dates. Thus, we concluded that diffusive transport of NO_3^- had a minor effect on the concentration decreases at the subsurface layer.

Reviewer's comment 3

2) Impact of the biogeochemistry and primary production

The authors concluded "This consumption could result in reduced new production in the subsurface layer after the bloom, when this layer would once again become part of the euphotic zone, if the diatoms sank to deeper layers." However, I cannot agree with this without the evidence that the diatoms are not increased

in this layer. Diatoms have some unique modes of nutrient uptake (Martin-Jezequel et al. 2000). Is the observed nutrient uptake of diatoms in the dark condition not linked to the growth? When the authors have the time-series chlorophyll a concentration data in the laboratory experiments, please show the data and discuss that they did not fix carbon. In the case of cyanobacteria, they grow up in the twilight zone (Sohrin et al. 2011). In addition, the dark condition in the laboratory may be different from the dark condition of the field. Even though the PAR is less than 0.1% at the surface, it was not completely dark in the ocean. Many exciting discussions may be possible: nitrate uptake (new production) may occur in the twilight zone but not contribute to the primary production/ new production is underestimated when the nitrate uptake is not measured in more dark layers.

Respond to the comment 3

We proposed alternative hypotheses (1 and 2) to deduce the influence of nutrient uptake by diatoms in dark subsurface layer in bloom.

1) If the diatom population that had consumed half of the nutrients in the dark subsurface layer sank to the deeper layer during the bloom, then the primary production in the subsurface layer after the bloom, at which time it would be part of the euphotic zone, would be reduced by half at maximum compared to the production in the case where there was no nutrient consumption during the dark period.

2) If the diatoms that had consumed nutrients in the dark subsurface layer remained in that layer after the bloom or migrated to the upper layer, they have a potential to rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom.

We need to test if diatoms that have once taken nutrients in darkness can grow rapidly in light afterward and if the light intensity (1% PAR, 0.1% PAR, and complete darkness) has an impact on nutrient uptake as future research. We would like to use Martin-Jezequel et al (2000) and Sohrin et al (2011) as references to plan the future research.

To clarify our viewpoints, we have revised as follows:

Line 433 - 472

Nutrient uptake by diatoms in dark subsurface layer after the peak of bloom would have impacts on primary production and distribution of phytoplankton in bloom and post-bloom. We propose alternative hypotheses (1 and 2) to deduce the influence of nutrient uptake in the dark subsurface layer.

1) If the diatom population that had consumed half of the nutrients in the dark subsurface layer sank to the deeper layer during the bloom, then the primary production in the subsurface layer after the bloom, at which time it would be part of the euphotic zone, would be reduced by half at maximum compared to the production in the case where there was no nutrient consumption during the dark period.
2) If the diatoms that had consumed nutrients in the dark subsurface layer remained in that layer after the bloom or migrated to the upper layer, they have a potential to rapidly grow under the returning light conditions when the euphotic zone deepened after the bloom.

In the case of Funka Bay, we note that the consumption of nutrients in the dark subsurface layer would have an impact outside the bay, because the subsurface water was exchanged with Oyashio water.

In relation to the second hypothesis, an interesting survival strategy for diatom, *Rhizosolenia*, which forms large aggregations (mats), has been proposed (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014). The survival strategy of *Rhizosolenia* is that they consume NO_3^- in the dark subsurface layer, and then migrate to the euphotic zone where they have a growth advantage in oligotrophic subtropical open ocean areas. For the coastal marine diatom, *Thalassiosira weissflogii,* was studied to examine changes in buoyancy in relation to ratios of carbohydrate to protein which determine the cell density (Richardson and Cullen, 1995). They revealed that accumulation of carbohydrate as a result of nitrate depletion leads rises in cellular density and sinking speed and that accumulation of protein as a

result of nitrate addition after the nitrate depletion leads a positive buoyancy. Several modelling studies have suggested contributions of primary production by vertically migrating phytoplankton to net primary production. For example, Witz and Lan Smith, (2020) estimated that vertically migrating phytoplankton contributes 7% of net primary production at the subarctic gyre of the western Pacific.

These previous studies have not yet found any evidence of decrease in NO_3^- in the dark subsurface layer from observation. If the hypothesis of diatoms' migration strategy proposed by previous studies is true, the results of our study will provide evidence for the decrease in NO_3^- in the dark subsurface layer associated with the diatoms' strategy.

Reviewer's comment 4

3) The structure of the manuscript

It was just an opinion, but I am familiar with the manuscript which divides results and discussion. I believe the authors can divide them. However, if the authors considered the present style is better, this is not mandatory. This is the option, too, but the title of the manuscript should be a more appealing one. The present title only attracts local interests. For example, when the authors consider the observed phenomenon possibly occurs everywhere under diatom blooms, the title can be revised as "Significant nutrient consumption in the dark subsurface layer during a diatom bloom: the case study on Funka Bay, Hokkaido, Japan".

Respond to the comment 4

According to the comment, we divided results and discussions and revised the title.

Reviewer's comment 5

L14: Times of observations are necessary. Technically, the authors' observation is not time-series, because the observation was conducted randomly.

Respond to the comment 5

We revised the manuscript accordingly as follows:

Line 479 - 480

We conducted repetitive observations in Funka Bay, Hokkaido, Japan, from 15 February to 14 April 2019 during and after the spring bloom.

Reviewer's comment 6

L21 "We believe that this is the first study to present observational evidence for the consumption of the main nutrients by diatoms in the dark subsurface layer during the spring bloom." In my opinion, this sentence is

not important. Instead of this sentence, the authors should add why they considered the nutrient decline does not occur by physical processes.

Respond to the comment 6

We added a sentence about exclusions of physical processes as an explanation of nutrient reduction in the subsurface layer. We removed the sentence “this is the first study to present ---” from abstract.

Line 22 - 23

We excluded possibilities of three physical process, water mixing, diffusive transport, and subduction, as reasons for the decrease in nutrients in the subsurface layer.

Reviewer’s comment 7

L26 “Si: NO3– ratio”. Yes, this is not wrong and described in Harrison et al 2004, but Si(OH)4:NO3 or Si: N ratio is more appropriate. This is just opinion.

Respond to the comment 7

We corrected accordingly.

Reviewer’s comment 8

L31–32: References are required.

Respond to the comment 8

We added a reference (Rosa et al., 2007).

Reviewer’s comment 9

L34–35: “From time-series observations in the bay, it is possible to examine the temporal changes of biochemical parameters within the same identified water mass while the water is in the bay.” I cannot understand this sentence clearly. What means “while the water is in the bay”?

Respond to the comment 9

We revised the sentence as follows:

Line 51 - 54

From repetitive observations in the bay, it is possible to collect seawater samples originated from the same water mass in different times when the water remains in the bay during the observation period and then examine the temporal changes of biogeochemical parameters within the same water mass.

Reviewer's comment 10

L37–39: "A massive spring bloom dominated by diatom species occurs in March every year before the Oyashio water flows into the surface of the bay, and it lasts until late March or early April, when Oyashio water occupies the surface of the bay (Odate 1987; Maita and Odate 1988)." I cannot understand this sentence. Please clarify. Can the author divide it into two sentences?

Respond to the comment 10

We revised the sentence as follows:

Line 56 - 59

In the Funka Bay, diatom bloom initiates in late winter, February, before Oyashio water flows into the bay (Kudo and Matsunaga, 1999). A massive spring bloom dominated by diatom species occurs in March every year (Odate 1987; Maita and Odate 1988) when Oyashio water flows into the bay. The bloom lasts until late March or early April when Oyashio water occupies the surface of the bay (Kudo and Matsunaga, 1999).

Reviewer's comment 11

L58–61: I could not find any meaning in these two sentences. I cannot see any discussion of VOIs in this manuscript. In addition, the reference is under consideration. So I considered these two sentences should be removed.

Respond to the comment 11

According to the comment, we revised as follows:

Line 51 - 56

From repetitive observations in the bay, it is possible to collect seawater samples originated from the same water mass in different times when the water remains in the bay during the observation period and then examine the temporal changes of biogeochemical parameters within the same water mass. For example, temporal changes in nutrients (Kudo and Matsunaga 1999; Kudo et al. 2000), dissolved iron (Hioki et al. 2015), volatile organic iodine (Shimizu et al. 2017) and isoprene (Ooki et al. 2019) have been examined in relation to primary production in the bay.

Reviewer's comment 12

Method: L72–73: "Observations in Funka Bay have been reported elsewhere (Shimizu et al. 2017)." What did the authors want to describe? Some information on the observations conducted in 2019 was described in 2017? Describe the details or remove the sentence.

Respond to the comment 12

According to the comment, we removed the sentence.

Reviewer's comment 13

L78: How do the authors calculate the analytical precision? This is very low. Did the authors measure the nutrient concentration of not-frozen samples? If the results are frozen samples, the precisions are too good, in particular, silicate.

Respond to the comment 13

We added information about the determination of precision as follows.

Line 91 - 93

Analytical precision was 0.12% for NO_3^-, 0.21% for NO_2^-, 0.19% for PO_4^{3-}, 0.11% for SiO_2, and 0.34% for NH_4^+ as determined by repeated measurement (n = 7) of reference seawater for nutrient standards (KANSO, standard Lot BZ, Osaka, Japan).

Reviewer's comment 14

L82: Where Thalassiosira nordenskioeldiicome from? Algae collection?

Respond to the comment 14

We added a sentence accordingly:

Line 100 - 101

Thalassiosira nordenskioeldii was isolated from natural seawater collected in the western subarctic Pacific Ocean in May 2019.

Reviewer's comment 15

L95: “We set the initial concentrations of nutrients at 23 times those of the first dark incubation.” Why did the authors set so high initial nutrient concentration? The environments are very different from the field observations.

Respond to the comment 15

We added information about the amount of added amount of nutrients as follows:

Line 312-314

The added amount of NO_3^- per chl-a (0.022 = 31.1 μmol L^{-1} / 1426 μg L^{-1}) was 6% of the ratio of NO_3^-/chl-a (0.40 = 4.8 μmol L^{-1} / 12 μg L^{-1}) in seawater at 40 m on 4 March.

Line 329-330

In the second experiment, we added excess amount of nutrients into the nutrient-depleted medium, in which cultured diatoms were in a decline phase of growth. The added amount of NO_3^- per chl-a (10.3 = 743.5 μmol L^{-1} / 72.5 μg L^{-1}) was 26 times of the seawater at 40 m on 4 March.

Reviewer’s comment 16

* Did not the authors conduct the microscopic observations? Is Thalassiosira nordenskioeldii the dominant species of the observations? This is very important. Because the other species is dominant, the authors’ incubation experiments are meaningless.

Respond to the comment 16

We added information about microscopic analysis as follows:

Line 308-311

From microscopic image analysis, *Thalassiosira nordenskioeldii* occupied 14.2% of number of phytoplankton cells (n = 1209) collected by plankton net (mesh = 100 μm) on 15 March 2019. Other dominant species were *Chaetoceros* spp. and other *Thalassiosira* sp. We confirmed that *Thalassiosira nordenskioeldii* was one of the dominant species in the spring bloom 2019.

Reviewer’s comment 17

L102–106: Please define the water masses at the materials and methods. When the authors defined in the materials and methods section, the results will be simpler.

Respond to the comment 17

We revised the manuscript accordingly.

Reviewer's comment 18

L106: "The revised classification result" This is unclear. Did the authors revise in this manuscript or revise in Ooki et al. 2019?

Respond to the comment 18

We used the definition of water mass classification proposed in Ooki e al. (2019). Ooki et al. (2019) made a minor change in the classification by Ohtani and Kido (1980).F

We removed the phrase "The revised classification result"

Reviewer's comment 19

L115–119: This is not a result. Please define in the materials and methods section.

Respond to the comment 19

We revised the manuscript accordingly.

Reviewer's comment 20

L123: "the original data in supplementary information of Ooki et al., (submitted)." Is it right? I can see the supplementary information of this manuscript.

Respond to the comment 20

We have used the same chl-a data in the two papers (this paper and unpublished paper). We removed the description "original data in supplementary information of Ooki et al., submitted". And, we added chl-a data in supplement material.

Reviewer's comment 21

L124: "The data for chl-a are taken from a related article." What does it mean? I think it is acceptable to share the data with other manuscripts.

Respond to the comment 21

We removed the description "The data for chl-a are taken from a related article".

Reviewer's comment 22

L150–168: These paragraphs were "discussion". These discussions can be put after the results section because the results after this paragraph are not contained the results of the discussion. For me, this style is hard to follow.

Respond to the comment 22

According to the comment, we moved these paragraphs from "results" to "discussion".

Reviewer's comment 23

3.3.2 This section (results of incubation experiments) should be shown before 3.3.1.

Respond to the comment 23

We revised accordingly.

Reviewer's comment 24

L270: Villareal et al. reported Rhizosolenia, and not Thalassiosira. Do the authors have any evidence on the vertical migration of Thalassiosira? If not, this discussion is speculative. In addition, the authors' names are wrong: Wirtz and Lan Smith (2020) are correct.

Respond to the comment 24

We added some explanations about the vertical migration of Thalassiosira (Richardson and Cullen, 1995). To clarify our viewpoint, we revised as follows:

Line 458-468

In relation to the second hypothesis, an interesting survival strategy for diatom, *Rhizosolenia*, which forms large aggregations (mats), has been proposed (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014). The survival strategy of *Rhizosolenia* is that they consume NO_3^- in the dark subsurface layer, and then migrate to the euphotic zone where they have a growth advantage in oligotrophic subtropical open ocean areas. For the coastal marine diatom, *Thalassiosira weissflogii,* was studied to examine changes in buoyancy in relation to ratios of carbohydrate to protein which determine the cell density (Richardson and Cullen, 1995). They revealed that accumulation of carbohydrate as a result of

nitrate depletion leads rises in cellular density and sinking speed and that accumulation of protein as a result of nitrate addition after the nitrate depletion leads a positive buoyancy. Several modelling studies have suggested contributions of primary production by vertically migrating phytoplankton to net primary production. For example, Witz and Lan Smith, (2020) estimated that vertically migrating phytoplankton contributes 7% of net primary production at the subarctic gyre of the western Pacific.

Dear anonymous referee2

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Best regards,

Corresponding author: Atsushi Ooki

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* We additionally carried out the dark incubation experiment using Thalassiosira culture with n = 4 samples.
*We additionally carried out the dark incubation experiment using natural seawater collected on 8 March 2022 before the peak of diatom bloom 2022.
*We revised the chapter structure.

Revised chapter structure

Response to reviewer 2

GENERAL COMMENTS

Reviewer's comment 1

*In general, I feel that the introduction is poorly structured and weak. A lot of background about the hydrology but also about the dynamic of the phytoplankton bloom and productivityin Funka Bay is missing. I invite the authors to develop their bibliography and look closer in the literature for studies that have already been conducted in Funka Bay and develop their introduction a little further. For example (and this is not exhaustive), Nakada et al. (2013) for water mass dynamics, Shinada et al. (1999) and Radiarta and Saitoh (2008) for phytoplankton bloom and productivity dynamics in the bay. Most importantly, the objectives of this work arenot clearly presented here. Why authors decided to focus on the processes affecting nutrient reduction in the dark subsurface layer during the bloom, what is the purpose of this study?

Response to the comment 1

According to the comment, we added a reference (Nakada et al 2013) and clarified the purpose of this study. We revised as follows:

Line 32-59

Dissolved iron and nitrate (NO_3^-) supplied from below the surface to the surface euphotic zone through winter vertical water mixing sustain spring phytoplankton bloom in the Oyashio region (Nishioka et al. 2011). Most previous studies about marine primary production have concerned with the nutrient consumption by phytoplankton in the euphotic zone because most phytoplankton species, except for dinoflagellates (e.g. Cullen and Horrigan 1981), are commonly assumed to be incapable of moving actively between the surface mixed layer and below the surface (subsurface layer). A few studies have focused on the vertical migration of a diatom, *Rhizosolenia*, to uptake nutrients in the subsurface layer and grow in the euphotic zone in the oligotrophic subtropical Pacific (Villareal et al. 1996; Richardson et al. 1998; Villareal et al., 1999; Villareal et al., 2014). As for the subarctic area, a modelling study that simulated a lot of chl-a profiles, taking into phytoplankton's migration behaviour, demonstrated that vertically migrating phytoplankton can pump up considerable amount of nutrient to the surface layer from the dark subsurface layer and contributes 7% of net primary production at the subarctic gyre of the western Pacific, Oyashio region (Witz and Lan Smith, 2020). These previous studies have not yet shown observational evidence of nutrient reduction associated with consumption by phytoplankton in the dark subsurface layer, however, nutrient reduction in the dark subsurface layer have been found in the Funka Bay, Hokkaido, Japan (Kudo and Matsunaga, 1999), which faces to the Oyashio-Kuroshio transitional area in the western North Pacific.

Oyashio water reaches the area off the coast of Hokkaido, Japan, where the subtropical water derived from Kuroshio or the Tsugaru warm current waters are also found (Rosa et al., 2007). A small portion of Oyashio water enters Funka Bay in early spring. The bay water exchanges twice a year, with cold Oyashio water in early spring and Tsugaru warm water in early fall (Ohtani 1971). The Oyashio water, a cold and low salinity water, flows into the bay along the northern coast of the bay, forming an anticlockwise flow from late of March to middle of April (Nakada et al. 2013). From repetitive observations in the bay, it is possible to collect seawater samples originated from the same water mass in different times when the water remains in the bay during the observation period and then examine the temporal changes of biogeochemical parameters within the same water mass. For example, temporal changes in nutrients (Kudo and Matsunaga 1999; Kudo et al. 2000), dissolved iron (Hioki et al. 2015), volatile organic iodine (Shimizu et al. 2017) and isoprene (Ooki et al. 2019) have been examined in relation to primary production in the bay. In the Funka Bay, diatom bloom initiates in late winter, February, before Oyashio water flows into the bay (Kudo and Matsunaga, 1999). A massive spring bloom dominated by diatom species occurs in March every

year (Odate 1987; Maita and Odate 1988) when Oyashio water flows into the bay. The bloom lasts until late March or early April when Oyashio water occupies the surface of the bay (Kudo and Matsunaga, 1999).

Line 74-77
In this paper, we examine the temporal variation of nutrient concentrations in Funka Bay from the early phase of the diatom bloom (February) to post-bloom (April) through repetitive observations in 2019. And we focused on the processes affecting nutrient reduction in the dark subsurface layer during the bloom to show evidence of nutrient consumption by diatoms in darkness.

Reviewer's comment 2
*Two main comments for the Material and Method section:
I do not think that 4 sampling days conducted in a random way can be considered as a time series, perhaps a survey…
Response to the comment 2
According to the comment, we used "repetitive" instead of "time-series".

Reviewer's comment 3
When investigating biological processes, incubation experiments such as those conducted in this study MUST be replicated (triplicates are a must), otherwise any interpretation coming outfrom the experiment results is only speculative and conclusion cannot be ensured. This is very important!
Response to the comment 2
According to the comment, we additionally carried out the third and fourth incubation experiments using the Thalassiosira culture additionally (each quadruplicated, n=4) and the natural seawater incubation experiments using seawater collected on 8 March 2022 (n = 3 or 4). The results of the third and fourth experiments were close to the nutrient reduction found at 30–50-m depths in Funka Bay. However, the dark consumption rates of our experiments and previous studies had wide range values. Thus, we concluded that "dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka bay".

We added sentences as follows:
Line 118-144
In the third and fourth *Thalassiosira* experiments, 40 mL of diatom culture was divided into two pre-culturing incubation bottles with 170 mL of modified f/2 medium (NO_3^-, 175 µmol L^{-1}; PO_4^{3-}, 6.5 µmol L^{-1}; $Si(OH)_4$, 18.8 µmol L^{-1}). For the third *Thalassiosira* experiments, the pre-culturing was done for 10 days; concentration of chl-a in the pre-cultured medium became 145 µg L^{-1} and concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ dropped as follows: NO_3^-, 9.27 µmol L^{-1}; PO_4^{3-}, 0.42 µmol L^{-1}; and $Si(OH)_4$, < 1 µmol L^{-1}. Relatively high concentrations of NO_3^- and PO_4^{3-} remained in the medium. For the fourth *Thalassiosira* experiments, the pre-culturing was done for 11 days; concentration of chl-a in the pre-cultured medium became 198 µg L^{-1} and concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ dropped as follows: NO_3^-, 0.66 µmol L^{-1}; PO_4^{3-}, 0.42 µmol L^{-1}; and $Si(OH)_4$, < 1 µmol L^{-1}. Nutrients had been rapidly consuming at the end of the pre-culturing on day 10 of the third experiment. We added nutrients (stock f/2 medium) into the pre-cultured mediums, after which concentrations (day 0 of dark incubations) were as follows: NO_3^-, 187 µmol L^{-1}; PO_4^{3-}, 7.66 µmol L^{-1}; and $Si(OH)_4$, 16.1 µmol L^{-1} for the third experiment; NO_3^-, 213 µmol L^{-1}; PO_4^{3-}, 9.08 µmol L^{-1}; and $Si(OH)_4$, 21.2 µmol L^{-1} for the fourth experiment. The 30 mL of each pre-cultured medium was used for chl-a and nutrient measurements. The remaining 160 mL of each pre-cultured medium was divided into 4 cell-cultivation flasks, and they were put in darkness at 6 °C. On days 1, 2, and 3, 8 mL and 1 mL of incubation medium (n = 4) were filtered to measure nutrient and chl-*a* concentrations, respectively.
For the natural seawater incubation experiment, we collected seawater at 5- and 40-m depths at the station 30 of Funka Bay on 8 March 2022. Fourteen of 200 mL seawater samples were collected in cell-cultivation flasks (11 flasks for 5-m depth water and 3 flasks for 40-m depth water), and they were

stored in a refrigerator for a day until treatment of the culture experiment. The concentrations of chl-a at 5- and 40-m depths were 14.3 and 9.09 μg L^{-1}, respectively. Three flasks of each depth water were put in a dark incubator at 5 °C for 12 days without nutrient addition (continuous dark (5 m / 40 m) in Table 1). Another 8 flasks of 5-m depth water were put under light condition (100 μmol photon s^{-1}, light : dark = 12hr : 12 hr) for 5 days at 5 °C (nutrient-deplete (5 m) in Table 1) to deplete nutrients; concentration of chl-a in the pre-cultured medium became 25.1 μg L^{-1} and concentrations of NO_3^-, PO_4^{3-}, and $Si(OH)_4$ dropped as follows: NO_3^-, < 0.05 μmol L^{-1}; PO_4^{3-}, < 0.05 μmol L^{-1}; $Si(OH)_4$, < 1 μmol L^{-1}. Then nutrients were added into the 4 flasks of the nutrient-deplete 5 m water, after which concentrations (day 0) were as follows: NO_3^-, 12.6 μmol L^{-1}; PO_4^{3-}, 0.38 μmol L^{-1}; and $Si(OH)_4$, 17.8 μmol L^{-1}. The other 4 of the 8 flasks of the nutrient-deplete 5 m water were not added nutrients. These 8 nutrient-depleted (5 m) incubation bottles were put in the dark incubator at 5 °C for 7 days. On days 0 and 7 on dark incubations, 8 mL and 10 mL of incubation medium were filtered to measure nutrient and chl-a concentrations, respectively.

Line 337-371

In the third and fourth *Thalassiosira* experiments, the added amount of NO_3^- per unit chl-a (0.77 – 0.96) was 1.9 – 2.4 times of the seawater concentration ratio at 40 m on 4 March. The results of the third and fourth experiments (Fig. 7a-h) demonstrated that the diatom culture consumed nutrients in darkness. The consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 3 of the dark incubation and the initial chl-a concentrations on day 0, were 0.034 – 0.043 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0059 – 0.0086 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.034 – 0.035 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–4.3 ~ –5.4 μmol L^{-1}), ΔPO_4^{3-} (–0.75 ~ –1.1 μmol L^{-1}), and $\Delta Si(OH)_4$ (–4.3 ~ –4.4 μmol L^{-1}) were close to the actual decreases between the two dates: ΔNO_3^-, –2.0 μmol L^{-1}; ΔPO_4^{3-}, –0.12 μmol L^{-1}; $\Delta Si(OH)_4$, –3.7 μmol L^{-1}. The chl-a concentrations were increased in darkness from 145 μg L^{-1} (day 0) to 250 μg L^{-1} (day 3) for the third experiment and from 198 μg L^{-1} (day 0) to 294 μg L^{-1} (day 3) for the fourth experiment.

In the natural seawater incubation experiment using seawater samples collected on 8 March 2022, the added amount of NO_3^- per unit chl-a (0.50 = 12.6 μmol L^{-1} / 25.1 μg L^{-1}) into nutrient-depleted seawater was within a range between the concentration ratio of NO_3^-/chl-a (0.33 = 4.9 μmol L^{-1} / 14.5 μg L^{-1}) in seawater at 5m and the ratio of 0.81 (= 7.4 μmol L^{-1} / 9.1 μg L^{-1}) at 40 m on 8 March 2022. The results of the natural seawater experiment, in which nutrients were added into the nutrient-depleted seawater after the pre-culturing under light condition, are shown in Fig. 8a-d. We found decreases in nutrient concentrations in the dark period after the nutrient addition. The consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 7 of dark incubation and the initial chl-a concentration on day 0, were 0.053 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0018 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.010 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–6.7 μmol L^{-1}), ΔPO_4^{3-} (–0.23 μmol L^{-1}), and $\Delta Si(OH)_4$ (–12.9 μmol L^{-1}) were greater than the nutrient decreases at the subsurface layer between 4 and 15 March 2019. On the other hand, results of the natural seawater experiment under continuous dark condition without nutrient-depletion by pre-culturing demonstrated that all nutrients were not consumed by phytoplankton in darkness (Fig. 8e-h). On 8 March 2022, the concentrations of chl-a and NO_3^- at 5 m were 14.3 μg L^{-1} and 4.6 μmol L^{-1} and those at 40 m were 9.2 μg L^{-1} and 7.4 μmol L^{-1}, respectively. The high level of chl-a in the surface (5 m) indicates the occurrence of the spring diatom bloom; and enough amount of NO_3^- (4.6 μmol L^{-1}) in the surface water suggests that phytoplankton were not under nutrient stress, that is, it was a growing phase before the peak of the bloom. We considered that phytoplankton collected from nutrient-repleted natural seawater on 8 March 2022 had not required nutrients in the dark culture environment.

The result of continuous dark incubation using natural seawater without pre-culturing, in which medium seawater had originally high concentration of NO_3^- (4.6 – 7.4 μmol L^{-1}) on 8 March 2022, was inconsistent with the result of the third *Thalassiosira* experiments, in which medium had the same level of NO_3^- (9.27 μmol L^{-1}) at the end of pre-culturing before nutrients were added. That is, nutrients were not consumed in continuous darkness by the natural seawater experiment but substantially consumed in darkness by the third *Thalassiosira* experiments. In the third *Thalassiosira* experiments, NO_3^- in the pre-cultured medium was rapidly consuming on day 10 of the pre-culturing

with approximately 8.61 μmol L^{-1} per day, which was calculated from the concentration difference of NO_3^- in pre-cultured medium on day 10 of the third experiment and day 11 of the fourth experiment: (9.27 – 0.66) μmol L^{-1} / (11 – 10) day. We considered that the rapid consumption of nutrients had been maintained in the dark period of the third *Thalassiosira* experiment.

Line 382-386

In the dark subsurface layer of Funka Bay between 4 and 15 March 2019, N-depleted diatoms sunk from the surface after the peak of bloom could have enhanced NO_3^- consumption in darkness. On the other hand, N-repleted diatoms in growing phase of bloom on 8 March 2022 would not have a potential to consume nutrients in the dark subsurface layer. Although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms after the peak of bloom had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay.

Reviewer's comment 4

*Section 3.2 of the Discussion is really long compared to other sections and contain a lot of redundancies. I suggest to re-organize this section chronologically instead of per nutrient, for example 3.2.1 pre-bloom, 3.2.2 bloom and 3.2.3 post-bloom situation. This might help reducingthe length of this section (especially for chl*a* and nitrate) and remove most of the redundancies.

Response to the comment 4

According to the reviewers', we have largely reconstructed the manuscript . We divided the chapter "results and discussion" into separate chapters.

Reviewer's comment 5

Moreover, this will allow authors to discuss the evolution of the nutrient ratios which will help confirming or infirming their hypothesis and which has not been explored in the discussion?

Response to the comment 5

According to the comment, we added a chapter as follows:

Line 266-276

3.3 Limiting factor of primary production during the bloom

On 15 February before the occurrence of massive diatom bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 9.1, 0.86 and 19.8 μmol L^{-1}, respectively. On 4 March at the peak of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.34, 0.43 and 5.6 μmol L^{-1}, respectively. Uptake ratios of N:P and Si:N at the surface between 15 February and 4 March were 20.5 {= (9.1 – 0.34) / (0.86 – 0.43)} and 1.62 {= (19.9 – 5.6) / (9.1 – 0.34)}, respectively. Similar uptake ratios during diatom bloom in Funka Bay have been reported to be N:P = 15.6 – 23.6 and Si:N = 1.9 – 2.7 (Kudo and Matsunaga 1999). From the uptake ratio of N:P, NO_3^- in the surface water could have been depleted since 4 March. On 15 March at the decline phase of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.54, 0.37 and 4.5 μmol L^{-1}, respectively. Since sufficient amount of $Si(OH)_4$ remained in the surface water on 15 March, we considered that the N-depletion in the surface water limited primary production after the peak of the bloom.

Line 382-383

In the dark subsurface layer of Funka Bay between 4 and 15 March 2019, N-depleted diatoms sunk from the surface after the peak of bloom could have enhanced NO_3^- consumption in darkness.

Reviewer's comment 6

*The conclusion is poorly reflecting the main observations and hypothesis raised by the authors to explain the nutrient drawdown observed in the dark subsurface water of Funka Bay. Indeed, Authors cannot reject the influence of vertical mixing as they did L284-288, they haven't discussed the possibility of nutrient diffusion (active or passive), and the consumption rate of diatoms measured during the incubation experiment are not enough to fully explain the observed nutrient drawdown. The conclusion MUST be revised to truly reflect the main results of this study.

Response to the comment 6

We added discussions about possibilities of diffusive transport of nitrate and entrainment of subducted water from the surface. And we revised the explanation about the results of incubation experiments.

Line 410 – 437

4.2.3 Diffusive transport between the surface and the subsurface layers

Third, we discuss an effect of diffusive transport of NO_3^- on concentration decrease at the subsurface layer (30 – 50 m) on 15 March. There is not any previous study to have measured diffusive coefficients (Kρ) in Funka Bay. We referred a range of Kρ (= 10^{-6} – 10^{-5} m^2 s^{-1}) measured just below the mixed layer (~ 30 m) at the western subarctic Pacific in summer (Dobashi et al. 2021). Concentration gradients of NO_3^- were –0.000221 μmol m^{-4} (= $\Delta NO_3^-{}_{20m–30m}$ / 10 m), –0.000141 μmol m^{-4} (= $\Delta NO_3^-{}_{30m–40m}$ / 10 m), –0.000115 μmol m^{-4} (= $\Delta NO_3^-{}_{40m–50m}$ / 10 m), and –0.0000135 μmol m^{-4} (= $\Delta NO_3^-{}_{50m–60m}$ / 10 m). The range of diffusive transport of NO_3^- were calculated to be 0.00022 – 0.0022 μmol m s^{-2} between 20 m and 30 m, which could result in concentration change of 0.021 ~ 0.21 μmol L^{-1} at 30 m for 11 days. Concentration changes between 30 m and 40 m and between 40 m and 50 m were calculated to be 0.013 ~ 0.13 μmol L^{-1} and 0.011 ~ 0.11 μmol L^{-1}, respectively. The sum of concentration changes at 30 m, which include transports from 20 m layer and 40 m layer, ranges from –0.20 μmol L^{-1} (= –0.21 + 0.013) to +0.11 μmol L^{-1} (= –0.021 + 0.13). Ranges of the sum of concentration changes at 40 m and 50 m were –0.12 ~ +0.096 μmol L^{-1} and –0.11 ~ –0.024 μmol L^{-1}, respectively. The observed decreases were of 1.6 μmol L^{-1} at 30 m, 2.0 μmol L^{-1} at 40 m, and 2.4 μmol L^{-1} at 50 m between these dates. Thus, we concluded that diffusive transport of NO_3^- had a minor effect on the concentration decreases at the subsurface layer.

4.2.4 Subduction of surface water into the subsurface layer

Fourth, we discuss a possibility if subduction of surface water caused the decrease in nutrient concentrations at the subsurface layer (30 – 50 m) of the observation station 30. At the medium depth (40 m) of the subsurface layer, temperature, salinity, and density were 3.5 – 3.6 °C, 33.64, and 26.7σ, respectively, on 4 and 15 March (Fig. 2). Suppose surface water in certain area of the bay subducted and it reached 40-m depth at the observation station on 15 March, the subducted water should have the same temperature, salinity, and density as it had been at the surface. The average current speed at 40-m depth between these dates was 3.3 cm s^{-1} (unpublish data), which was obtained from acoustic doppler current profiler (ADCP) set on the sea floor at the station. The middle layer water at the station could have reached from anywhere of the bay within 11 days. We obtained the spatial distributions of temperature, salinity, and density at the sea surface (1 m) on 4 March using the ocean reanalysis product provided by Meteorological Research Institute in Japan (Fig. 9a-c).
From these spatial distributions, there was not any area that satisfied required temperature (3.5 – 3.6 °C), salinity (33.64), and density (26.7σ) to form subduction water at 40-m depth of the observation station, see an enlarged map of Fig. 9c. We considered that the subsurface layer water at the station was not associated with subduction. Thus, we excluded a possibility of subduction as a reason for the nutrient decline.

Line 163-171

2.5 Spatial distributions of temperature, salinity, and density at the sea surface

Spatial distributions of temperature, salinity, and density at the sea surface (1 m) were obtained from an ocean reanalysis product provided by Meteorological Research Institute in Japan. This is produced

with an operational system for monitoring and forecasting the status of coastal and open-ocean waters around Japan (the JPN system; Hirose et al., 2020). The JPN system includes a double-nested ocean model, the core of which is a Japanese coastal model with a horizontal resolution of 2 km. Three sub-models are interconnected using a nesting technique: a global model (horizontal resolution ~100km), a North Pacific model (horizontal resolution ~10km), and Japanese coastal model (horizontal resolution ~2km). A four-dimensional variational method is applied to the North Pacific model as the assimilation scheme. The process of tides and river runoff are taken into consideration in this JPN system. See the technical report for more detail (Hirose et al. 2020).

Line 478-505

Conclusion

We conducted repetitive observations in Funka Bay, Hokkaido, Japan, from 15 February to 14 April 2019 during and after the spring bloom. We found reductions in nutrient concentrations in the dark subsurface layer both before and after the peak of the bloom and concluded that the latter reduction was caused by dark consumption by diatoms that had grown in the euphotic zone and then sank to the dark subsurface layer. We reached this conclusion using the following rationale.

(1) From the dark incubation experiments, we confirmed that the diatom *Thalassiosira nordenskioeldii*, which is one of the dominant diatom species in the bloom of Funka Bay, could consume nutrients in darkness at substantial rates and that phytoplankton in nutrient-depleted natural seawater collected in the bay before the peak of diatom bloom on 8 March 2022 could also consumed nutrients in darkness. Although the consumption rates varied over a wide range, we concluded that dark consumption of nutrient by diatom that had been growing at the surface and then sank into the subsurface layer had a potential to reduce nutrient by half in the dark subsurface layer (30–50 m).

(2) We excluded water mixing, diffusive transport, and subduction as possible reasons for nutrient reduction in the subsurface layer between 4 March and 15 March. First, the stratification between the surface and subsurface layers was strengthened after 4 March, and therefore we considered vertical mixing of water between the layers to be limited. The small decline in salinity at 30 m and no change in salinity at 40–50 m means that mixing with low-salinity Oyashio water could not explain the nutrient reduction, even if the Oyashio water had no nutrients. Second, we estimated the diffusive transport of NO_3^- to have a minor effect on concentration decrease at the subsurface layer. Third, we showed that there was not any area that satisfied required surface temperature, salinity, and density to form subduction water at the subsurface layer (medium depth of 40 m) at the observation station on 15 March 2019. Thus, we excluded the possibilities of subduction as a reason for the nutrient reduction.

The consumption of nutrients in darkness has been studied in many simulated in-situ incubation experiments, with the goal of understanding dark consumption during a daily cycle within the euphotic zone. We believe that this is the first study to demonstrate observational evidence of consumption of the three main nutrients (NO_3^-, PO_4^{3-}, and $Si(OH)_4$) by diatoms in the dark subsurface layer during a bloom. This consumption could result in reduced new production in the subsurface layer after the bloom, when this layer would once again become part of the euphotic zone, if the diatoms sank to deeper layers. Further research is needed examining the survival strategies of diatoms consuming nutrients in the dark subsurface layer.

Reviewer's comment 7

ABSTRACT

The abstract should use non-technical terms. For example, while "mixed-layer" is a common term used in oceanography and it is ok to use it in the abstract, Dark Zone Depth needs to be defined. Another option could be use "below the euphotic zone" instead of "dark zone depth".The main

conclusion of the abstract needs to be revised since the nutrient consumption by diatom in the dark layer is likely not the only possible explanation to the observed nutrient drawdown in the subsurface waters of Funka Bay.

Response to the comment 7

According to the comment, we added a definition “dark-zone depths (0.1% of surface PAR depth) “ in the abstract. We defined the euphotic-zone depth and the dark-zone depth as 1% and 0.1%, respectively, of surface PAR depth. Thus, we used the term, “dark-zone depth”.

We added explanations that the physical processes were excluded from reasons of the nutrient reduction.

Line 22-23

We excluded possibilities of three physical process, water mixing, diffusive transport, and subduction, as reasons for the decrease in nutrients in the subsurface layer.

Reviewer’s comment 8

L14 and later in the manuscript: February 15th to April 14th of 2019. Please be careful on the calendar notation along the manuscript.

Response to the comment 8

Professional scientific editors (ELSS, Inc., Tsukuba, Japan) recommended to use “15 February to 14 April”. If the BG editor requires proof reading by professional editors for the revised manuscript, we will offer proof reading to ELSS again.

Reviewer’s comment 9

L16: “On both date”? During the whole time series from Feb 15th to Apr 14th? or during the bloom from March 4th to March 15th? By the way, winter water was above the euphotic zone (so above the dark-zone depths) on March 4th in fig.2.

Response to the comment 9

We have revised as “on 4 and 15 March”.

Yes, winter water was found above the euphotic zone depth on 4 March. Deeper winter water remained below the dark-zone depths from 4 March to 15 March.

Reviewer’s comment 10

L17: Same comment as above, please specify which dates. On fig.4, while NO_3^- concentration decreases by approximately half, it is absolutely not the case for $Si(OH)_4$ and PO_4^{3-} (e.g. at 50m concentrations dropped from 0.7 to 0.6 µmol L^{-1} and from 15 to 13 µmol L^{-1} for PO_4^{3-} and $Si(OH)_4$ respectively)

Response to the comment 10

According to the comment 9, we specified the two dates (4 and 15 March) at the prior sentence, thus “these dates” is specified as “4 and 15 March”.

Reviewer’s comment 11

L20-23: Authors present a list of several publications that have already studied dark nutrient consumption rates and ratios (see L250-259)

Response to the comment 11

According to the comment, we removed the sentence from abstract.

Reviewer's comment 12
L25-26: Surface euphotic zone sounds redundant.
Response to the comment 12
According to the comment, we removed "surface" form "surface euphotic" throughout the manuscript.

Reviewer's comment 13
L28: Use either "Si:N ratio" or "$Si(OH)_4$:NO_3^- ratio"
Response to the comment 13
According to the comment, we used "Si:N ratio".

Reviewer's comment 14
L38: It is actually believed that the intrusion of the Oyashio water in the Bay triggers the diatombloom by strengthening water column stratification.
Response to the comment 14
We recognize that diatom growth begins in late winter before the Oyashio water flows into the bay and massive diatom bloom in the bay is triggered by intrusion of the Oyashio water.
According to the comment, we revised as follows:
Line 56-58
In the Funka Bay, diatom bloom initiates in late winter, February, before Oyashio water flows into the bay (Kudo and Matsunaga, 1999). A massive spring bloom dominated by diatom species occurs in March every year (Odate 1987; Maita and Odate 1988) when Oyashio water flows into the bay.

Reviewer's comment 15
L56-58: This is unfortunate since nutrient limitation can change from time to time. This manuscript doesn't have to focus on nutrient limitation in the surface water, however, it would be interesting to have a paragraph (even a short one) about what controls the dynamic of the bloom in the euphotic zone during this study. This is important since nutrient drawdown occurring in the surface layer will likely affect the subsurface biogeochemistry by creating gradient (and potentially diffusive fluxes) of nutrients, or by triggering adaptive response from the biology (such as vertical migration, resting spore formation etc.)
Response to the comment 15
According to the comment, we added a chapter about nutrient ratio and nutrient limitation as follows:

Line 266-276

3.3 Limiting factor of primary production during the bloom

On 15 February before the occurrence of massive diatom bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 9.1, 0.86 and 19.8 μmol L^{-1}, respectively. On 4 March at the peak of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.34, 0.43 and 5.6 μmol L^{-1}, respectively. Uptake ratios of N:P and Si:N at the surface between 15 February and 4 March were 20.5 {= (9.1 – 0.34) / (0.86 – 0.43)} and 1.62 {= (19.9 – 5.6) / (9.1 – 0.34)}, respectively. Similar uptake ratios during diatom bloom in Funka Bay have been reported to be N:P = 15.6 – 23.6 and Si:N = 1.9 – 2.7 (Kudo and Matsunaga 1999). From the uptake ratio of N:P,

NO_3^- in the surface water could have been depleted since 4 March. On 15 March at the decline phase of the bloom, the average concentrations of NO_3^-, PO_4^{3-} and $Si(OH)_4$ at the surface 0 – 10 m were 0.54, 0.37 and 4.5 µmol L^{-1}, respectively. Since sufficient amount of $Si(OH)_4$ remained in the surface water on 15 March, we considered that the N-depletion in the surface water limited primary production after the peak of the bloom.

Reviewer's comment 16

L58-61: VOI are not presented nor discussed in this manuscript. Please remove these sentencesor add a figure, present and discuss the results of VOI measurements, and highlight their contribution in answering the objectives of the present study (why VOIs are measured?).

Response to the comment 16

According to the comment, the reference about VOI was removed from there.

Reviewer's comment 17

L72-73: What does this sentence mean? What kind of observations? Are they relevant for this paper? Please remove this sentence or develop.

Response to the comment 17

According to the comment, we removed the sentence.

Reviewer's comment 18

L77: SiO_2 is not a nutrient. This is the formula for particulate silica, silicic acid (the nutrient) is $Si(OH)_4$, please make sure to double-check the notations throughout the manuscript.

Response to the comment 18

According to the comment, we changed "SiO_2" to "$Si(OH)_4$" throughout the manuscript.

Reviewer's comment 19

L78: Analytical precision: this is almost too good to be true! I am curious to see how this has been calculated?

Response to the comment 19

We measured reference seawater for nutrient standards (KANSO). The precisions of repetitive analysis are pretty good.

According to the comment, we added an explanation about the precision as follows:

Line 91-93

Analytical precision was 0.12% for NO_3^-, 0.21% for NO_2^-, 0.19% for PO_4^{3-}, 0.11% for $Si(OH)_4$, and 0.34% for NH_4^+ as determined by repetitive measurement (n = 7) of reference seawater for nutrient standards (KANSO, standard Lot BZ, Osaka, Japan).

Reviewer's comment 20

L83: please use axenic instead of sterile. F/2 medium has approximately 106 µmol.L^{-1} $Si(OH)_4$ 882 µmol.L^{-1} NO_3^-, and 36 µmol.L^{-1} PO^{3-} (see Guillard and Ryther, 1962; Guillard, 1975 or Andersen et al (2005), Algual Culturing Techniques). It is more likely a modified (not particularlySi-rich) f/2 medium then, although I agree it is still plenty of nutrients for diatoms.

Response to the comment 20

According to the comment, we used "axenic" instead of "sterile" and added a word "modified".

Reviewer's comment 21

L86: This is really unfortunate since checking for contamination is crutial regarding the objectiveof the incubation experiment. Indeed, contamination by a population of other micro-algae or bacteria can affect the consumption (or recycling) of NO_3^- and PO_4^{3-} (although it is probably less the case for $Si(OH)_4$). This have to be mentioned and discussed in the discussion.

Response to the comment 21

According to the comment, we added an explanation about the bacterial contamination as follows:

Line 316-318

Since we did not check the bacterial contamination after the experiment, bacterial consumption and/or recycling of nutrients in the culture might have an effect on the results. We assumed that the bacterial activity had a less effect on nutrient changes in the high-density diatom culture.

We could not check bacterial contamination after the culture experiment. Instead, we additionally conducted natural seawater incubation experiments using seawater collected on 8 March 2022. The results of natural seawater experiments would include an effect of bacterial activity.

Line 346-371

In the natural seawater incubation experiment using seawater samples collected on 8 March 2022, the added amount of NO_3^- per unit chl-a (0.50 = 12.6 μmol L^{-1} / 25.1 μg L^{-1}) into nutrient-depleted seawater was within a range between the concentration ratio of NO_3^-/chl-a (0.33 = 4.9 μmol L^{-1} / 14.5 μg L^{-1}) in seawater at 5m and the ratio of 0.81 (= 7.4 μmol L^{-1} / 9.1 μg L^{-1}) at 40 m on 8 March 2022. The results of the natural seawater experiment, in which nutrients were added into the nutrient-depleted seawater after the pre-culturing under light condition, are shown in Fig. 8a-d. We found decreases in nutrient concentrations in the dark period after the nutrient addition. The consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 7 of dark incubation and the initial chl-a concentration on day 0, were 0.053 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0018 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.010 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–6.7 μmol L^{-1}), ΔPO_4^{3-} (–0.23 μmol L^{-1}), and $\Delta Si(OH)_4$ (–12.9 μmol L^{-1}) were greater than the nutrient decreases at the subsurface layer between 4 and 15 March of 2019. On the other hand, results of the natural seawater experiment under continuous dark condition without nutrient-depletion by pre-culturing demonstrated that all nutrients were not consumed by phytoplankton in darkness (Fig. 8e-h). On 8 March 2022, the concentrations of chl-a and NO_3^- at 5 m were 14.3 μg L^{-1} and 4.6 μmol L^{-1} and those at 40 m were 9.2 μg L^{-1} and 7.4 μmol L^{-1}, respectively. The high level of chl-a in the surface (5 m) indicates the occurrence of the spring diatom bloom; and enough amount of NO_3^- (4.6 μmol L^{-1}) in the surface water suggests that phytoplankton were not under nutrient stress, that is, it was a growing phase before the peak of the bloom. We considered that phytoplankton collected from nutrient-repleted natural seawater on 8 March 2022 had not required nutrients in the dark culture environment.

The result of continuous dark incubation using natural seawater without pre-culturing, in which medium seawater had originally high concentration of NO_3^- (4.6 – 7.4 μmol L^{-1}) on 8 March 2022, was inconsistent with the result of the third *Thalassiosira* experiments, in which medium had the same level of NO_3^- (9.27 μmol L^{-1}) at the end of pre-culturing before nutrients were added. That is, nutrients were not consumed in continuous darkness by the natural seawater experiment but substantially consumed in darkness by the third *Thalassiosira* experiments. In the third *Thalassiosira* experiments, NO_3^- in the pre-cultured medium was rapidly consuming on day 10 of the pre-culturing with approximately 8.61 μmol L^{-1} per day, which was calculated from the concentration difference of NO_3^- in pre-cultured medium on day 10 of the third experiment and day 11 of the fourth experiment: (9.27 – 0.66) μmol L^{-1} / (11 – 10) day. We considered that the rapid consumption of nutrients had been

maintained in the dark period of the third *Thalassiosira* experiment.

Reviewer's comment 22

L93-97: What was the purpose of this second dark incubation? and why adding much morenutrient compare to the first one? Please explain.

Response to the comment 22

Silicic acid was almost depleted on day 2 of the first experiment. If the diatoms consumed $Si(OH)_4$ much more quickly than we collected the culture sample on day 2, the daily consumption rates are underestimated. So, we added much more nutrients in the second experiment.

According to the comment, we added explanations as follows:

Line 325-329

Silicic acid was almost depleted on day 2 of the dark incubation. If the diatoms exhausted $Si(OH)_4$ earlier than we collected the culture sample on day 2, the daily consumption rates are underestimated.

In the second *Thalassiosira* experiment, we added excess amount of nutrients into the nutrient-depleted medium, in which cultured diatoms were in a decline phase of growth.

Reviewer's comment 23

3.1. Hydrographic features

Are the water masses defined based only on salinity? Or did the authors use a T-S diagram to define the distribution (depth range) of the different water masses during the study? If so, it would have been useful to have a figure with a T-S diagram that illustrate the position of watermass end-members (such as in Shimizu et al. 2017) in supplement material at least.

Response to the comment 23

According to the comment, we added an illustration of water mass definition as supplement material.

Reviewer's comment 24

L111: "biogeochemical"

3.2 Biogeochemical parameters

My guess is that authors meant "biogeochemical" instead of "biochemical" since biogeochemical refers to processes associated nutrient cycles and nutrient distribution whilebiochemical is more related to intra-cellular processes.

Response to the comment 24

According to the comment, we used "biogeochemical" instead of "biochemical".

Reviewer's comment 25

L115-118: Why authors chose this definition of the mixed layer, please explain AND cite appropriate reference. Same comment for euphotic zone and dark depth zone, appropriatereferencing is missing.

Response to the comment 25

According to the comment, we added references and explanations why we adapted the threshold 0.125 kg m^{-3} as follows:

Line 154-162

The surface mixed layer was defined as the layer in which density differences ($\Delta\sigma$) were within 0.125 kg m^{-3} relative to the density at 5-m depth. The threshold $\Delta\sigma$ = 0.125 kg m^{-3} is often used for monthly

mean of mixed layer in oceanic climate studies (Spall 1991), while the threshold $\Delta\sigma$ = 0.01 kg m^{-3} is used for snap-shot observations (Thomson and Fine 2003). We used the maximum threshold $\Delta\sigma$ = 0.125 kg m^{-3} to ensure that the subsurface layer water had not mixed with the surface layer during intervals (11 days to a month) between our observations. The euphotic-zone depth was defined as the depth at which photosynthetically active radiation (PAR) was 1% of the surface PAR, where amount of photosynthesis is equal to respiration (Marra 2004). We defined the dark-zone depth at which PAR was 0.1% of the surface PAR, where amount of photosynthesis is approximately tenth part of the 1% PAR depth taking into account the light intensity only as a limiting factor of photosynthesis.

Reviewer's comment 26

L118: in general terms like "much less" should be avoided in papers, please be more specific and give a threshold or a range.

Also, mixed layer, euphotic zone and dark depth zone can be defined in the previous paragraph,since they can be considered as hydrographic features as well.

Response to the comment 26

According to the comment, we revised the sentence. Please see the response to the comment 25.

Reviewer's comment 27

L120-124: Authors cannot discuss in detail data that are not available either in another ALREADY published work, in a database, in supplement or in the main text of the manuscript.Authors cannot refer to an unpublished work when it concerns data availability, especially when these data are used to make some of the figures. This is not acceptable! Moreover, I don't understand this sentence since the chl*a* profile in fig.3 looks pretty low and homogenous on Feb 15 and it seems that the chl*a* data ARE presented in the supplement…

Response to the comment 27

We confirmed that the chl-a data have been presented in the supplement. And we removed the description about the supplement of unpublished paper. From the supplement data of this paper, the chl-a profile on 15 Feb is not homogeneous: higher concentrations (0.65 – 1.35 μg/L) at 0 – 70 m, and lower concentrations (< 0.05 μg/L) at 75 – 95 m.

Reviewer's comment 28

L125-126: 27 to 30 μg L^{-1} of chl*a* sounds very high to me. It looks like it's between 1.5 and 2 times higher than what is usually found in the bay (see L126-128). Is it a particularly exceptional year? Why is chl*a* that high?

Response to the comment 28

Yes, chl-a concentrations of 27 to 30 μg L^{-1} are very high. We will examine the mechanism of such a high chl-a event as future research.

Reviewer's comment 29

L131: Sinking particles and suspended particles are different things. What makes authors conclude that particles can be sinking vs. in suspension? Please develop.

Response to the comment 29

We can not make a distinction between sinking particle and suspended particle. We used "sinking" in the manuscript.

Reviewer's comment 30
L140: A reference for the annual maximum is missing.
Response to the comment 30
The annual maximum level (~20 μmol L^{-1}) of nitrate was found in the bottom water in summer (e.g. Kudo et al. 2007).
According to the comment, we removed a phrase "the annual maximum".

Reviewer's comment 31
L150-167: Since there is a section specifically discussing the nutrient consumption in the dark subsurface layer, I would suggest to remove those hypotheses here to avoid redundancy with section 3.3. and to add this material to the discussion in section 3.3. This section 3.2 could thusbe renamed "nutrient dynamic in the euphotic zone and dark subsurface layer" or something similar.
Response to the comment 31
We have largely reconstructed the manuscript. We divided the chapter "results and discussion" into separate chapters.

Reviewer's comment 32
L168-175: I do not see why the first explanation of vertical mixing suggested for March 4th NO_3^- decrease at depth cannot be still valid in March 15th. Indeed, authors based their explanation mostly on the AOU profile. However, AOU profile in the 30-50m layer in March 14th looks prettymuch the same as in March 4th (see fig.3).
Response to the comment 32
The reason for the same AOU values in the subsurface layer between 4 and 15 March is that net O_2 production had not occurred there.
According to the comment, we added AOU profiles (Fig. 5f) to show that the values stayed unchanged between the two dates. And we changed a phrase "are slightly decrease" to "remained almost the same values".

Reviewer's comment 33
L180-186: Note that the signal of regeneration is also clearly seen from the strongly positivevalues of AOU at depth in April 14th.
Response to the comment 33
According to the comment, we added a sentence about AOU increase in the bottom water as follows:
Line 253-254
Obvious increase of AOU in the deep water (80 – 95-m depth) was found from 15 March (average 20.9 μmol L^{-1}) to 14 April (average 56.0 μmol L^{-1}), see Fig. 4b and Fig. 5f.

Reviewer's comment 34
L185: It is important to be more specific here. For example, the sentence can be "The very high PO_4^{3-}, $Si(OH)_4$ and NH_4^+ concentrations measured at depth (1.9, 26.1, and 3.2 μmol L^{-1}, respectively) clearly indicate that nutrient regeneration already occurred at the bottom of the water column in April 14th".
Response to the comment 34
According to the comment, we added explanations about rises in bottom water concentrations in detail

as follows:
Line 234-236
In the deep water (80–95 m), the NO_3^- concentrations slightly increased with time since 15 March: 5.38 μmol L^{-1} on 4 March, 5.26 μmol L^{-1} on 15 March, and 6.60 μmol L^{-1} on 14 April.
Line 251-257
In contrast to the subsurface layer, the average concentrations of PO_4^{3-} and $Si(OH)_4$ in the deep layer (80 – 95-m depth) increased with time; 0.78 μmol L^{-1} and 15.3 μmol L^{-1} on 4 March, 0.89 μmol L^{-1} and 22.3 μmol L^{-1} on 15 March, and 1.57 μmol L^{-1} and 25.1 μmol L^{-1} on 14 April, respectively. Obvious increase of AOU in the deep water (80 – 95-m depth) was found from 15 March (average 20.9 μmol L^{-1}) to 14 April (average 56.0 μmol L^{-1}), see Fig. 4b and Fig. 5f. Because the obvious increase of PO_4^{3-} coincided with the rise in AOU, it likely resulted from remineralization following the decomposition of organic matter suspended in the bottom water or settled on the seafloor. The increase of $Si(OH)_4$ in the bottom water is likely resulted from dissolution of biogenic silica settled on the seafloor.
Line 261-265
Because the NH_4^+ concentrations were at their lowest during winter with total column average of 0.25 μmol L^{-1} on 15 February, the signal from remineralization could be clearly detected on 15 March with average of 0.54 μmol L^{-1} at the subsurface water (30 – 50 m). The deep water NH_4^+ concentrations obviously increased with time since 4 March: 0.31 μmol L^{-1} on 4 March, 0.95 μmol L^{-1} on 15 March, and 3.05 μmol L^{-1} on 14 April.

Reviewer's comment 35
3.3 Nutrient consumption in the dark subsurface layer
I suggest to add a subsection between subsection 3.3.1 and 3.3.2 to discuss a third hypothesisfor nutrient drawdown in the dark subsurface layer, the diffusive flux of nutrient, that have been neglected so far in the manuscript but I think could be of significant importance.
Response to the comment 35
We added a discussion about the diffusive transport of nitrate. Please see the response to the comment 6.

Reviewer's comment 36
L211: It is currently impossible to see the density difference between 5 and 30m in fig. 4 since both density profiles are incomplete. Moreover, it is more about the shape or curvature of the density profile rather than the magnitude of the density difference. Indeed, a smooth and progressive gradient of density will not act as a strong barrier against exchange compared to asharp change in density (even though this latter is smaller).
Response to the comment 36
According to the comment, we added CTD profile (Fig.2) to show all plot and used the density gradient instead of the magnitude of density difference.
Line 391-392
Because the density (σ) gradient between 20-m and 30-m depths, ($\sigma_{30m} – \sigma_{20m}$) / (30 m – 20 m), 0.0033 (kg m^{-3} m^{-1}) on 4 March substantially increased to 0.021 (kg m^{-3} m^{-1}) on 15 March,

Reviewer's comment 37
L212-214: Please show these data in a figure
Response to the comment 37
The data can obtain from the JMA website. We added a URL of download site as follows:
Line 345

https://www.data.jma.go.jp/risk/obsdl/index.php

Reviewer's comment 38
L215: Although this might be true, I do not agree with authors since we do not have access to the observations that support this conclusion (there is no figure illustrating the wind speed andthe density profiles on fig.4 are incomplete).
Response to the comment 38
According to the comment, we revised the figure (CTD profiles in Fig. 2) to show all plots.

Reviewer's comment 39
L218-228: This could be another subsection focused on discussing horizontal advection
Response to the comment 39
We added a discussion about a possibility of subduction water (subducted water horizontally moved to the observation station). Please see the response to the comment 6.

Reviewer's comment 40
L222-224: A mixing ratio of 8:2 between Funka-Bay and Oyashio waters is a curious way to present the mixing between these two water masses. Won't it be clearer to say that to generate the observed decrease in salinity, a mixing with 25% of Oyashio water (or 1:4 Oyashiowater and Funka Bay water) is necessary. By the way, by using salinities of 33.0, 33.47 and 33.58 for Osahio water, Funka Bay water, and the mix between the two of them, respectively; my estimate is 20% (1:5) of Oyashio water needed to produce the observed decrease in salinity.
L227-228: This is not because the vertical mixing cannot explain all the nutrient drawdown that this hypothesis should be excluded, it is more likely not the main driver of the nutrient drawdown. It doesn't mean that it is not happening, it is more likely a combination of different processes. I suggest to rephrase this conclusion.

Response to the comment 40
According to the comment, we revised the sentence as follows:
Line 403-405
A mixing between 20% of Oyashio water and 80% of Funka Bay water at 30 m would change the salinity at 30-m depth from 33.58 (on 4 March) to 33.47 (on 15 March).

Reviewer's comment 41
L231-232: Same comment as above. The nutrient reduction in the subsurface layer is more likely driven by multiple processes, consumption of nutrient by diatom in the dark might be oneof them (not the only one).
Response to the comment 41
We agree with the comment 41 that the nutrient reduction in the subsurface layer is driven by multiple processes. We estimated that the diffusive transport occupied approximately 10% of the reduction and concluded that the consumption by diatom had the most important effect on the reduction. Please see the response to the comment 6.

Reviewer's comment 42
L237 and after: Please explain the μg chl*a*$^{-1}$ d^{-1} unit used for consumption rates. Chl*a* concentrations during the incubation experiment are not presented in fig.5 nor elsewhere in the manuscript. Also, in the main text NO_3^-, PO_4^{3-} as well as Si(OH)$_4$ consumption are discussedbut only NO_3^- is show in fig.5.

The evolution of ALL nutrient concentration MUST be presented somewhere if authors are discussing them in the manuscript.

Response to the comment 42

According to the comment, we added an explanation of the consumption rate and the values of chl-a concentrations in the figure. We used the chl-a concentrations on day 0 for the calculations of nutrient consumption rate per unit chl-a.

And, we added figures of the dark incubation results for PO_4 and $Si(OH)_4$ and added a sentence explaining chl-a changes during the dark incubations.

Line 318-320

The daily consumption rates per unit chl-a amount calculated from the concentration difference of nutrients between day 0 and day 2 and the initial concentration of chl-a (1426 μg L^{-1}) of the dark incubation

Line 344-345

The chl-a concentrations were increased in darkness from 145 μg L^{-1} (day 0) to 250 μg L^{-1} (day 3) for the third experiment and from 198 μg L^{-1} (day 0) to 294 μg L^{-1} (day 3) for the fourth experiment.

Revised figures: Fig. 6 and Fig. 7 (attached below)

Reviewer's comment 43

L238-342: Although calculation of consumption rates cannot be verified since nutrient concentration during the incubation are not presented, it seems that the consumption rate of diatoms estimated from the incubation experiment are very low and cannot fully explain the nutrient drawdown observed in the 30-50m layer between march 4th and March 14th.

Response to the comment 43

According to the comments, we additionally conducted the third and fourth experiments. We explained that "although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay" as follows:

Line 337-344

In the third and fourth *Thalassiosira* experiments, the added amount of NO_3^- per unit chl-a (0.77 – 0.96) was 1.9 – 2.4 times of the seawater concentration ratio at 40 m on 4 March. The results of the third and fourth experiments (Fig. 7a-h) demonstrated that the diatom culture consumed nutrients in darkness. The consumption rates, which were calculated from the concentration difference of nutrient between day 0 and day 3 of the dark incubation and the initial chl-a concentrations on day 0, were 0.034 – 0.043 μmol (μg chl-*a*)$^{-1}$ d^{-1} for NO_3^-, 0.0059 – 0.0086 μmol (μg chl-*a*)$^{-1}$ d^{-1} for PO_4^{3-}, and 0.034 – 0.035 μmol (μg chl-*a*)$^{-1}$ d^{-1} for $Si(OH)_4$. The estimated ΔNO_3^- (–4.3 ~ –5.4 μmol L^{-1}), ΔPO_4^{3-} (–0.75 ~ –1.1 μmol L^{-1}), and $\Delta Si(OH)_4$ (–4.3 ~ –4.4 μmol L^{-1}) were close to the actual decreases between the two dates: ΔNO_3^-, –2.0 μmol L^{-1}; ΔPO_4^{3-}, –0.12 μmol L^{-1}; $\Delta Si(OH)_4$, –3.7 μmol L^{-1}.

Line 384-386

Although the dark consumption rates had wide ranges, we concluded that dark consumption by diatoms after the peak of bloom had a potential to reduce nutrients by half in the dark subsurface layer of Funka Bay.

Reviewer's comment 44

L243-245: Are these consumption rates calculated between day 0 and day 2 such as in the first incubation experiment? I don't think rates from the second incubation experiment can be compared to rates in the first experiment since too many parameters were different between the two experiments (e.g. concentrations were much higher in the second experiment, since diatom uptake usually follows a Michaelis-Menten function, this can change a lot the dynamic of the diatom uptake!). Does this mean that, because the nutrient concentrations in the field were much lower compare to those set up during both experiments, we can assume that the nutrient consumption rates were probably much lower in the water column and thus would probably contribute to a very small amount of the observed nutrient drawdown?

Response to the comment 44

We revised the time interval for the consumption rate calculation of the second experiment to between day 0 and day 2. We added explanations about the amount of added nitrate per unit chlorophyll. The observed nitrate amount per unit chlorophyll in the bay were within the range of added nitrate amount per unit chlorophyll in cultures of dark incubations.

Line 312-314

The added amount of NO_3^- per unit chl-a (0.022 = 31.1 μmol L^{-1} / 1426 μg L^{-1}) was 6% of the concentration ratio of NO_3^-/chl-a (0.40 = 4.8 μmol L^{-1} / 12 μg L^{-1}) in seawater at 40 m on 4 March.

Line 329-330

The added amount of NO_3^- per unit chl-a (10.3 = 743.5 μmol L^{-1} / 72.5 μg L^{-1}) was 26 times of the seawater concentration ratio at 40 m on 4 March 2019.

Line 337-338

In the third and fourth *Thalassiosira* experiments, the added amount of NO_3^- per unit chl-a (0.77 – 0.96) was 1.9 – 2.4 times of the seawater concentration ratio at 40 m on 4 March.

Line 346-349

In the natural seawater incubation experiment using seawater samples collected on 8 March 2022, the added amount of NO_3^- per unit chl-a (0.50 = 12.6 μmol L^{-1} / 25.1 μg L^{-1}) into nutrient-depleted seawater was within a range between the concentration ratio of NO_3^-/chl-a (0.33 = 4.9 μmol L^{-1} / 14.5 μg L^{-1}) in seawater at 5m and the ratio of 0.81 (= 7.4 μmol L^{-1} / 9.1 μg L^{-1}) at 40 m on 8 March 2022.

Reviewer's comment 45

L247-249: I do not understand this sentence. Why would the consumption rate be largely dependent on the chl*a* content in the diatom cells if, as suggested by authors L165, this nutrientconsumption occurs "without photosynthetic growth"? Please explain.

Response to the comment 45

Although we did not count cell number in the seawater sample collected in the bay and the sample of incubation experiment, capacity of nutrient consumption in darkness might depend on the cell density and nutrition conditions for each cell. We will examine property of nutrient consumption in darkness associated with chl-a, cell number, nutrition condition of diatom cell as future research.

Reviewer's comment 46

L248-249: I totally agree with this statement!

Response to the comment 46

The main purpose of this study is to show the observational results of nutrient reduction in the dark subsurface layer associated with consumption by diatom, excluding possibilities of physical processes. Investigations of dark consumption properties associated with nutrition condition of diatom cell are remained for future research.

Reviewer's comment 47
L251: I do not understand this sentence, please rephrase.
Response to the comment 47
We revised the sentences as follows:
Line 372-374
Cochlan et al. (1991) carried out onboard incubations with a diatom dominating natural seawater setting dark periods of 2–4 hours after light periods. They have reported dark consumption rates for NO_3^- of 0.09–0.14 μmol (μg chl-*a*)$^{-1}$ d^{-1}, which are close to the results from our second incubation.

Reviewer's comment 48
L268-273: All diatoms do not migrate. Those vertical migrations have been observed for mats of Rhizosolenia. Do authors have evidence for such a behavior in Thalassiosira? This needs to be discussed here.
Response to the comment 48
According to the comment, we added a reference about the changes in buoyancy of Thalassiosira (Richardson and Cullen, 1995).
Line 461-465
For the coastal marine diatom, *Thalassiosira weissflogii,* was studied to examine changes in buoyancy in relation to ratios of carbohydrate to protein which determine the cell density (Richardson and Cullen, 1995). They revealed that accumulation of carbohydrate as a result of nitrate depletion leads rises in cellular density and sinking speed and that accumulation of protein as a result of nitrate addition after the nitrate depletion leads a positive buoyancy.

Reviewer's comment 49
L275: What are those assumptions? They need to be presented and discussed here.
Response to the comment 49
According to the comment, we revised the sentence as follows:
Line 470-472
These previous studies have not yet found any evidence of decrease in NO_3^- in the dark subsurface layer from observation. If the hypothesis of diatoms' migration strategy proposed by previous studies is true, the results of our study will provide evidence for the decrease in NO_3^- in the dark subsurface layer associated with the diatoms' strategy.

Reviewer's comment 50
L279-280: Once again, I'm not convinced that 4 sampling days can be considered as a time series.
Response to the comment 50
We used "repetitive" instead of "time-series" in the revised manuscript.

Reviewer's comment 51
L291-292: I do not agree with this. The nutrient consumption rates estimated from the incubation experiment are too low to explain the nutrient drawdown observed in the 30-50m layer in Funka Bay.
Response to the comment 51
We carried out additional incubation experiments. The results were close to the observed nutrient reduction rate per chlorophyll. Please see the response to the comment 43.

Reviewer's comment 52
Figure 1: There is only one station/sampling site in this study. Describe what O (Oyashio water)and T (Tsugaru water) are in the figure caption or legend.
Response to the comment 52
According to the comment, we described "T: Subtropical Tsugaru current" and "O: Subpolar Oyashio current" in the figure legend.

Reviewer's comment 53
Figure 2: Define the euphotic zone and MLD in the captions instead of in the legend. Be consistent in the formatting of the legend (e.g. WO: Transitional water instead of TransitionalWater: WO).
Response to the comment 52
According to the comment, we described the definition of the euphotic-zone and mixed-layer depths in the caption and corrected the legends, "WO: Transitional Water".

Reviewer's comment 54
Figure 3: MLD is not at the same depth on Apr 14th in fig.2 (40m) and fig.3 (15m), same on March 4th fig.2 (~5m) vs. fig.3 (~10m). Please remove the red rectangle that is supposed to show the concentration change in 4-15 March. It actually doesn't help to read the figure. Most importantly this figure involves a lot of interpolations made from a total of four stations. The time coverage is probably not enough and could generate bias in the figure that could lead to misinterpretation. For example, on panel a. it seems that the bloom started soon after the sampling in Feb15, and peaked on March 4th, but we know that diatoms can consume nutrients pretty quickly in the mixed layer and the bloom could have started anytime between Feb 15th and March 4th, same for the termination of the bloom. I would suggest to present these data as profiles which will be more accurate instead of interpolated time-sections.
Response to the comment 54
We think that the rectangle showing the depth-temporal range (30-50m, 4-15 March) helps to read the figure. According to the comment, we added figures of vertical profile of chl-a, nutrients and AOU on (Fig. 5) to avoid the misinterpretation. We corrected the MLD on Apr 14th in the figure and revised the sentence as follows:

Line 203-204
15 March Chl-*a* concentrations had decreased at all depths by 15 March, however, there were still high levels (0–10 m, 11.0–16.2 μg L^{-1}) within the euphotic surface mixed layer (0 – 18 m) and in the deeper dark layer (20–95 m, 2.3–7.8 μg L^{-1}).

Reviewer's comment 55
Figure 4: The scale on the x axis in the temperature, salinity and density panels are not appropriate since the profile corresponding to March 15th in all panels is cut at the surface, same for the March 4th density profile. It is hard to discuss incomplete profiles. Right now, wecannot conclude that stratification is stronger on March 15th as stated L211.
The nutrient concentration difference in panel d, e and f are pretty obvious indeed, no need to add extra arrows. Moreover, the left-pointing arrow on panel c doesn't mean anything since density increases in the mixed layer from March 4th to March 15th. I would be interesting to add the standard deviations for the nutrient concentration measurements.
Response to the comment 55

According to the comment, we revised the CTD profiles (Fig.2) to show all and remove the arrows. Although we determined the analytical precision of nutrient measurements by repetitive analysis of standard seawater (KANSO), we did not measure the seawater sample more than three times a sample. Thus, we could not show the standard deviation on the figure.

Reviewer's comment 56

Figure 5: Overall, I like this figure. It is clear, although the scale of the x axis on panel b does notmake sense (two squares correspond to 4 days (0 to 4) then to two days (4 to 6), then back to 4days (6 to 10). I also still don't understand how the authors have defined their set-up conditions(e.g. why adding 30µmol. L^{-1} NO_3^- in the first experiment and around 750µmol L^{-1} in the second one? Why this threshold of 1429µg L^{-1} chl*a* in the first experiment but 72.5µg L^{-1} in the second one? Although I don't remember is the culture experiment has been replicated (Working with biology, I think it is crucial to run that kind of experiments in triplicate, or at least duplicate them) but this figure needs error bars.

Response to the comment 56

According to the comment, we revised the figure. The initial concentrations of chl-a were the results of pre-cultivation for growing diatom till nutrients were once exhausted in the light condition. According to the comment, we carried out additional experiments (n = 4). We revised the figures of the first and second experiments and added figures of the additional third and fourth experiments.