Response to review Report#1

Reviewer's comment #1-1)

The authors described "diatoms that had been growing in the surface waters and then sank to the subsurface layer" (L25–26). However, any evidence did not show the growth in the surface layer. In addition, the authors did not reject the effect of vertical migration of diatoms. Therefore, I cannot clearly understand what the authors want to say here. I feel that this sentence is not essential. The authors described that pre-incubation is necessary for nutrient consumption of diatom in the dark (is it right? Please explain clearly in the results section), and the fact Please clarify.

Response to the comment #1-1)

According to the comment, we removed the description "~~that had been growing in the surface waters and then sank to the subsurface layer.~~" and revised the sentences to explain the fact as follows.

Abstract, Line 19-25:

Incubation experiments using the diatom *Thalassiosira nordenskioeldii* showed that this diatom could consume added nutrients in the dark at substantial rates after the pre-culturing to deplete nutrient. Incubation experiment using natural seawater collected in growing phase of bloom on 8 March 2022 also showed that nutrient-depleted phytoplankton could consume added nutrients in the dark. We excluded possibilities of three physical process, water mixing, diffusive transport, and subduction, as the main reasons for the decrease in nutrients in the subsurface layer. We conclude that the nutrient reduction in the subsurface layer (30–50 m) between 4 and 15 March 2019 could be explained by dark consumption by diatoms at that layer.

Conclusions, Line 484-489:

(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 added nutrients in the dark at substantial rates after preculturing to deplete nutrients 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 added nutrients in

the dark. Although the consumption rates varied over a wide range, we concluded that dark consumption of nutrient by diatom at the dark subsurface layer had a potential to reduce nutrient by half in the dark subsurface layer (30–50 m).

Reviewer's comment #1-2)

2) The structure of the manuscript must be improved again. I could not find the results of "incubation experiments." I confused the descriptions of the "without pre-culturing" experiment. I could not find the explanations of the "without pre-culturing" experiment in the materials and methods sections, so I cannot understand what this indicate.
The results section is hard to follow. The description is fragmentary. In particular, at 3.2 Biogeochemical parameters.

Response to the comment #1-2)

According to the comment, we have revised the structure of the manuscript.
1) We described the results of incubation experiments in the Result chapter.
2) We changed the description from "without pre-culturing" to "without nutrient addition"
3) We removed meaningless descriptions from the incubation results.

~~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 dark by the natural seawater experiment but substantially consumed in the dark 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.~~

4) We removed meaningless descriptions that had made fragmentary and hard to follow from the chapter 3.2 "Biogeochemical parameters".

3.2.2 Nitrate

.....~~In this subsurface layer, AOU remained almost the same values between 4 and 15 March, suggesting that there was not any influence from photosynthetic O_2 production and therefore no photosynthetic NO_3^- consumption.~~ We hypothesized that the diatoms that had settled from the surface to the subsurface layer consumed NO_3^- in the dark. This possibility will be discussed in section 4.2. The NO_3^- concentrations in the deeper layer (60–95 m) had not changed since 4 March.

14 April By 14 April, the euphotic-zone depth had deepened to 47 m. The NO_3^- concentrations had decreased to below the detection limit (<0.05 μmol L^{-1}) in the upper euphotic zone (0–30 m) and decreased to 1.4 μmol L^{-1} in the lower euphotic zone (40 m). ~~Because the influence of Oyashio water extended from the surface to the subsurface layer, these decreases occurred simultaneously with the water exchange. It is possible that the NO_3^- concentration in the original Oyashio water had already been diminished by diatom consumption before the water entered the bay.~~ In the deep water (80–95 m), the NO_3^- concentrations slightly increased from 5.26 μmol L^{-1} on 15 March to 6.60 μmol L^{-1} on 14 April. ~~There is a time lag for regeneration of NO_3^- in bottom water after organic matter decomposition because the regeneration of NO_3^- follows the remineralization of NH_4^+ from organic matter and its oxidation (nitrification). A time lag of 1–2 months for NO_3^- regeneration after NH_4^+ regeneration has been observed every year in Funka Bay (Kudo et al. 2007). Thus, the signal from NO_3^- regeneration could not be seen during the spring bloom, and a slight signal was detected on 14 April. In contrast, signals of the regeneration of PO_4^{3-} and NH_4^+ from organic matter in bottom water were obvious on 15 March and 14 April, after the decline phase of the bloom, as discussed in the next two sections.~~

Reviewer's comment #1-3)

3) To reject the effect of the physical processes, the authors use the ocean circulation model. Please check temperature and salinity in the model is the same as in the observations (should be added as supplement information). Is the model localized for Funka Bay? If the T-S is the same as in the observation, I feel the authors cannot reject the possibility of physical processes without the gridded observations. Of course, the authors can be the effect of diatom consumption in the dark condition may be the primary cause based on the quantitative estimation of the incubation experiments.

Response to the comment #1-3)

According to the comment, we added modelled vertical profiles of temperature, salinity, and density on 4 and 15 March 2019 around the station 30 in Funka Bay in supplementary figure (Fig. s3). The model results were not good agreement with the observational results. We also explained about it.

Line 432-437: Note that the modelled vertical profiles of temperature, salinity and density were not in good agreement with the observed profiles (Fig. s3). In the model result, the influence of low salinity Oyashio water, which flowed in the surface of Funka Bay, was estimated to be stronger than the observational result. If the influence of Oyashio water had been as strong as the model result, the subsurface water (30 – 50 m) might have changed between 4 and 15 March 2019. We considered that the subsurface water had stayed between both dates because of weak Oyashio water inflow.

Reviewer's comment #1-4)

4) Please check the usages of parentheses and tilde (~). Tilda means similarity and does not mean "range."

Response to the comment #1-4)

According to the comment, we revised the manuscript not to use '~' as range.

Line 412 – 417: 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 from –0.12 to +0.096 μmol L^{-1} and from –0.11 to –0.024 μmol L^{-1}, respectively.

Response to review Report#2

Reviewer's comment #2-1)

The dynamics of primary production and associated nutrient consumption in the ocean surface layer have focused on the processes in the euphotic layer, where sufficient light is available for photosynthesis. In this study, the authors discuss various aspects on the apparent nutrient utilization in the twilight zone, where light is not sufficient for photosynthesis.
I have serious concern on the logics that nutrient consumption occurs in the field by defining the twilight layer as the dark layer.
The first thing to consider is that in oceanography, the lower limit of the euphotic layer is defined as the layer that attenuates to 1% of the surface PAR. On the other hand, the surface PAR varies widely in time and space with the changes with the cloud cover and solar incidence angle. The surface PAR under clear skies may exceed 2000 μE m-2 sec-1, and the calculated PAR at the bottom of the euphotic layer is 20 μE m-2 sec-1. On cloudy days, the surface PAR drops below 200 μE m-2 sec-1, and the PAR at the bottom of the euphotic layer is 2 μE m-2 sec-1. On the other hand, the compensating light intensity at which photosynthesis and respiration balance in the photosynthesis-light curve should have a certain value ranging from 1.2 μE m-2 sec-1 to 30 μE m-2 sec-1, depending on the literature, calculated from 24-hour values (Marra, 2004; Regaudie-de-Gioux & Duarte, 2010). It seems possible that the light condition exceeds the compensation light intensity at depths below the euphotic layer during the daytime under clear skies in the field, whereas the compensation light intensity of T. nordenskioeldii is not known. Furthermore, the evaluation of compensation light intensity for net production should be done on a daily (24-hour) basis, not on an instantaneous basis, as in the oxygen-based net production study by Gran (1912). Compensation light intensity is also expressed in terms of 24-hour integrated photon flux. This is because the positive production during the daytime is balanced by the negative production during the nighttime, when respiration exceeds production. In other words, photosynthesis may take place during the daytime even below the euphotic layer and the associated nutrient uptake may occur although nutrient uptake does not always synchronize photosynthesis. Thus, it is not appropriate to consider that nutrient uptake occurs in the absence of photosynthesis below the euphotic layer. In addition, the sense of the balance of carbon dioxide and oxygen in photosynthesis and respiration can not apply to the uptake of nutrients in the metabolic cycle of phytoplankton. In other words, in respiration, phytoplankton does not produce nutrients. The author should revise the present manuscript considering this point.

Response to the comment #2-1)

According to the comment, we clearly defined the dark-layer depth, which does not mean aphotic layer, as follows.

Line 164-166: We defined the dark-layer 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.

And we added vertical profile of ratio of PAR relative to the surface PAR and temporal variation of the surface PAR obtained from the Muroran meteorological observatory to show the light environment during the bloom. Please see the added figure (Fig. 2d) and supplementary figure (Fig. s2). We added explanations about light environment. According to the referee's comment, we used compensation light intensity expressed by 24-hour average (Regaudie-De-Gioux and Duarte, 2010) to compare the light intensity at the dark-layer depth during the bloom in Funka Bay. We assumed that photosynthesis below the dark-layer depth (less than 0.1% of surface PAR) made no difference in biochemical parameters such as nutrients.

Line 190-198: As for light environment, euphotic-zone depths (or compensation depth), which are defined as the depth where PAR was 1% of the surface PAR, were 40 m on 15 February, 11 m on 4 March, and 17 m on 15 March. Dark-layer depths, which we defined as the depth where PAR was 0.1% of surface PAR, were 60 m on 15 February, 17 m on 4 March, and 30 m on 15 March (Fig. 2d). The daily average of surface PAR during the period between 4 March and 15 March including day and night was 19.3 mol photon m^{-2} d^{-1} (= 224 μmol photon m^{-2} s^{-1}), which was estimated from the global solar radiation at the Muroran meteorological observatory (Fig. s2). The daily average of PAR at the dark-layer depth was estimated to be 0.0193 mol photon m^{-2} d^{-1}, which was only 1.8% of global average of compensation irradiance (1.1 mol photon m^{-2} d^{-1}) for metabolic balance, photosynthesis = respiration (Regaudie-De-Gioux and Duarte, 2010) Below the dark-layer depths, we assumed that photosynthesis made no difference in biochemical parameters described in latter sections.

We toned down the expression about photosynthesis at the dark layer, as follow.

Line 355-357: The latter reduction could not have been affected by photosynthetic consumption by diatoms because there was almost no light available for photosynthesis. Here we discuss the possible reasons for the nutrient reductions between 4 and 15 March.

According to the comment " In other words, in respiration, phytoplankton does not produce nutrients", we removed the following description, because nutrients could be consumed when AOU did not change at compensation depth.

~~In this subsurface layer, AOU remained almost the same values between 4 and 15 March, suggesting that there was not any influence from photosynthetic O_2 production and therefore no photosynthetic NO_3^- consumption.~~

Reviewer's comment #2-2)

L309: In assessing dominant phytoplankton, the use of plankton net (100 μm mesh) may cause a failure in collecting smaller phytoplankton even diatom species. It seems OK for just collecting T. nordenskioeldii for culture experiment.

Response to the comment #2-2)

We also think that the use of plankton net (100 μm mesh) is OK just for collecting aggregates of T. nordenskioeldii for culture experiment.

Reviewer's comment #2-3)

The authors should cite Kudo et al. (2015) in ECSS. They conducted annual primary production measurement in Funka Bay by the in situ 24-hour mooring incubation.

Response to the comment #2-3)

According to the comment, we cited the latest paper which reported annual primary production in Funka Bay (Kudo et al., 2015), as follows.

Line 63-64: One-third of annual primary production occurs during the spring bloom (Kudo and Matsunaga 1999; Kudo et al. 2015).