Reply to comments

This study aims to demonstrate in the Kuroshio area near northern East China Sea, that turbulence-induced nitrate flux can stimulate phytoplankton production in this seemingly oligotrophic ocean, while microzooplankton respond quickly to graze down the phytoplankton. As a consequence, high phytoplankton biomass is not observable. The authors used turbulence and nitrate sensor to demonstrate the nitrate flux, use nutrient enrichment experiments to demonstrate effects of nitrate flux on phytoplankton growth, and dilution experiments to measure microzooplankton grazing. This work is really interesting and deserves publishing in Biogeosciences. I have following comments that aim to help improve this manuscript.

>We really appreciate your kind comments to our findings. We indicated point-by-point response to the following comments. Hopefully, these are enough responses to your comments and suggestions.

Main concerns:

1. Potential effects of microzooplankton:phytoplankton ratio on the enrichment experiments: Table 1 shows that the chl-a and microzooplankton standing stock at the beginning of the incubations varied. The relative abundance of microzooplankton to phytoplankton may change the strength of top-down control. I wonder if adding microzooplankton:chl-a ratio or standing stock of microzooplankton and chl-a density to the regression analysis (Figure 5) can further explain the variation of phytoplankton growth after enrichment.

   >Your comments are really great and thanks. As you mentioned, we computed correlation between the slope of phytoplankton growth rates to the nutrients gradients and micro-sized heterotrophs biomass. Because no significant correlation was found for any size fractions to micro-sized heterotrophs, we have deleted these results from the manuscript. However, since some readers might have similar point of your view, we added these results in Figure 5 and some descriptions in the revised manuscript as follows.

   "The slope of a linear regression between growth rates of the size-fractionated chlorophyll and the logarithms of the nitrate enrichments at each incubation provided a metric of the sensitivity of their growth rates to nutrient supply (Supplement Fig 1). To explain why growth rates of the size-fractionated chlorophyll varied among stations, the slopes were compared to the nitrate+nitrite (Fig 5a) and phosphate concentrations (Fig 5b) and microzooplankton biomass (Fig 5c) in the ambient seawater without enrichment. No significant correlation was found for all size-fractionated chlorophyll to the micro-sized heterotrophs biomass. On the other hand, there was a negative correlation of the slopes for all size-fractions to the nitrate plus nitrite or phosphate concentrations, indicating that the stimulation of their growth rates by nutrients supply was greater for all size-fractionated chlorophyll under more oligotrophic conditions."

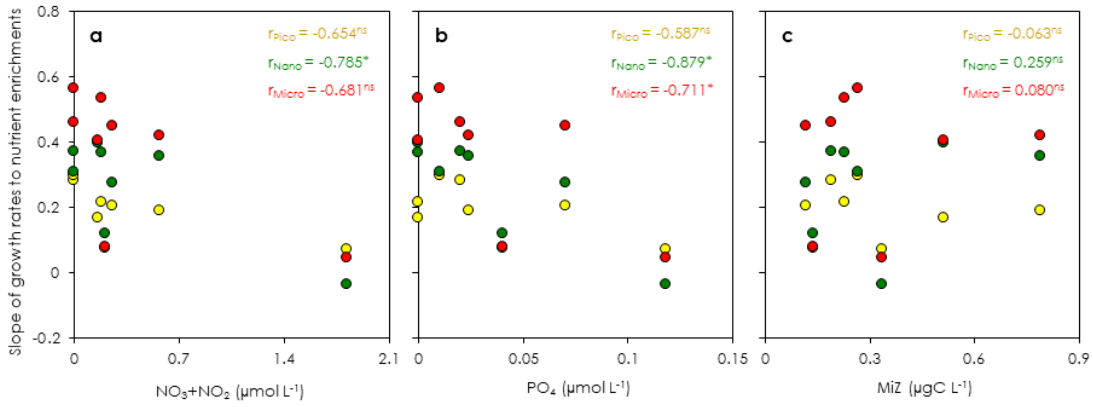

Fig 5. (Kobari et al.)

2. Enrichment experiments that did not exhibit clear effect of ambient nutrient on phytoplankton growth enhancement to enrichment (Lines 161-167 and Figure 5): Indeed there is a negative trend between phytoplankton growth-enrichment regression slope and [NO3−+NO2−] or [PO43−] in control experiments. However, the plankton communities that have small regression slopes and low r2 (r2<0.5; F01 and K08 in Fig. 5 and Table 1, which I labeled in the figure below) experienced quite different in situ nutrient condition, and only K08 seems to drive the negative trend. I would like to know if the negative trend remains after removing these two sets of low-r2 points. Furthermore, is there any possible explanation why the two incubations under low and high nutrient concentration reacted similarly to nutrient enrichment?

>We appreciate nice comments. As you suggested, correlation coefficients are much low (−0.014 to −0.778) and no significant if the slope of the phytoplankton growth rates at both stations are removed from Figure 5. According to the results in Table 2, phytoplankton growths at both stations tended to be higher than those at the other stations and positive even under no enrichment, particularly for micro-sized phytoplankton. We reported that larger phytoplankton predominated in coastal waters were often entrapped in frontal eddies and meanders of the Kuroshio around the study sites and advected into the Kuroshio (Kobari et al. 2019, Geophysical Monograph 243: 223-243). Probable explanations are that growths of phytoplankton communities at both stations are already stimulated with the advected coastal waters before our bottle experiments and nutrients are consumed for those phytoplankton communities particularly at K08. There is no evidence to support such hypothesis, however, we could not add further explanations in the revised manuscript.

3. “Intra-guild” predation within microzooplankton community (Line 158-160): The results indicate that enrichment slightly increased the growth rate of nauplii but not always increase ciliate growth, especially when enrichment is low. According to the biomass change of the three types of microzooplankton to enrichment, the increase of nauplii is not as significant as ciliates when enrichment is high (Figure 3). I think, maybe the intraguild predation of ciliates by nauplii inhibit the growth of ciliates when ciliate growth enhanced by low enrichment was not strong enough to compensate their mortality by nauplii feeding. As the enrichment increase further, fast growing ciliates can outgrow the consumption by large nauplii that grow and react more slowly to environmental change, and thus ciliate growth and biomass accumulation increase. If the body size ratio between nauplii and ciliates in the incubations fit the predator-prey mass ratio of nauplii (Hansen et al. 1994), this is possible to happen.

   >We appreciate your kind suggestions to our results. It might be another possibility. Based on our data sets, the ratio of mean equivalent spherical diameter of body mass between copepod nauplii (88 μm) and naked ciliates (16 μm) was estimated to be 5:1 and much different from to the predator-prey mass ratio (i.e., 18:1) reported by Hansen et al. (1994). As described above, no significant correlation was found for the growth response of phytoplankton to nutrients gradients. We think that such intraguild predation of copepod nauplii on naked ciliates would not happen in the bottles. However, we added such explanations in the revised manuscript as follows.

   “On the other hand, “intra-guild” predation within micro-heterotrophs community might be another explanation on the less clear pattern of their standing stocks and growth rates. Growth rates of copepod nauplii were always higher than those of naked ciliates, especially under no or less nitrate supply. The ratio of mean equivalent spherical diameter of body mass between copepod nauplii (88 μm) and naked ciliates (16 μm) was estimated to be 5:1 and much different from to the predator-prey mass ratio (i.e., 18:1, Hansen et al., 1994). Thus, such intraguild predation of copepod nauplii on naked ciliates would not happen in the bottles. More importantly to no or less clear pattern of the growth of micro-heterotrophs, the results from the simultaneously conducted experiments imply that phytoplankton productivity is stimulated by the turbulent nitrate flux and rapidly grazed by microzooplankton but standing stocks and growths of micro-heterotrophs are not elevated during 3 days in the Kuroshio Current. Increase of micro-heterotrophs standing stocks and their trophic transfer to mesozooplankton might be found in the further downstream of the Kuroshio Current.”

4. Stoichiometry of nutrient supply in Kuroshio (Lines 82-83): The enrichment and dilution experiments supplied phytoplankton with nitrate and phosphate molar concentration in 15:1 ratio (slightly N-limited, relative to the Redfield ratio 16:1). Did this ratio mimic the inorganic N:P concentration ratio or N:P flux by turbulent mixing in Kuroshio? Since this study focus on the nitrate supply from turbulent mixing, I expect that N should be limited. Nevertheless, I would like to know more about the stoichiometric condition of this study area and its potential effect on phytoplankton growth.

   >You are right. As you can find in Table 1, the ratios of nitrate/nitrite and phosphate molar concentrations showed N-limited conditions at ambient waters excepted for one station. As reported by Hasegawa et al. (2019, Geophysical Monograph 243: 191-205), 15:1 was measured in the ECS-Kuroshio and defined for the stoichiometric ratio of nutrients enrichment in our bottle experiments. On the other hand, in my

knowledge, no information on the stoichiometric effects on phytoplankton growth is available in the ECS-Kuroshio.

5. I will appreciate data to demonstrate the accuracy of in situ nitrate sensor (e.g. comparing with measurements using water collected by sampling bottles). This issue is particularly important when nitrate concentration is low in the water.

>Thanks. The measurement methodology for in situ nitrate sensor is just published in Japanese journal (Hasegawa et al. 2019, Bull Coast Oceanogr 27: 59-64). We added some explanation as follows referring the previous report.

"The nitrate sensor was calibrated with the observed nitrate concentrations (accuracy: 0.37 mmol m-3, Hasegawa et al. 2019)."

6. English needs substantial polishing to ensure correct grammar and wording. Some sentences are difficult to understand.

>Thanks for suggestion. We checked all phrases in the manuscript again and revised the incorrect grammars and words.

Editorial comments:

Abstract:

I have concerns on "rapid trophic transfer" in the title. The authors show evidence of rapid microzooplankton consumption of phytoplankton, but did not show evidence of trophic transfer. Suggested title: "Phytoplankton growth and consumption by microzooplankotn stimulated by turbulent nitrate flux suggest rapid trophic transfer in the oligotrophic Kuroshio

>Yes, we agreed and revised the title as you suggested.

The writing of Abstract is confusing. Readers cannot tell what are the results obtained from the experiments, what are the results from other studies, and what are the inferences from those results. I think these issues need to be clearly clarified in Abstract.

Line 29: I cannot understand this sentence, and what the authors intend to say.

>This sentence is revised as follows.

"Even though vulnerable life stages of major foraging fishes have a risk to be entrapped by frontal eddies and meanders and encountered under the low food availability, they have life cycle strategies to grow and recruit around the Kuroshio Current."

Line 31: This conclusion sentence is inference based on the results and should be written as so.

>Agree. We revised the phrase like this.

"Here we report that phytoplankton growth and consumption by microzooplankton is stimulated by turbulent nitrate flux amplified with the Kuroshio Current."

Line 35: "were simulated"

>Thanks. We revised.

Line 35: “Results of dilution …

>Thanks. We added.

Line 37: Please explain what you mean by "invisible".

>Yes, we wanted to mean “phytoplankton and microzooplankton productivity have long been undetectable by satellite images and oceanographic observations”. Since the readers might be confused, however, we deleted “invisible”.

Introduction

Line 40: I cannot understand what is "originates to".

>Thanks. We revised this phrase like this.

“The Kuroshio enters the East China Sea from the east of Taiwan and flows along the continental slope until it passes through the Tokara Strait into the western North Pacific (Fig 1a).”

Line 43: In spite of such “seemingly” unproductive

>Yes, we added.

Line 46: I cannot understand this sentence.

>Thanks for comments. We revised as follows.

“Highly vulnerable early life stages of many foraging species have a risk to grow and recruit under the oligotrophic and unproductive waters in the ECS-Kuroshio (hereafter called the “Kuroshio Paradox”: Saito, 2019), even if the warm temperatures of the Kuroshio Current could enhance cellular metabolic processes and then growth.”

Methods:

Line 78: Please explain the motivation of using nutrient gradient in experiment in this paragraph, so that the readers can follow the logic flow better.

>We mentioned the motivation just before this sentence. However, as you suggested, we explained the motivations for EXPa and EXPb just before the section of “Experimental setup” as follows.

“Two different types of bottle incubations were performed in the present study. For phytoplankton and micro-heterotrophs growth rates in response to in situ nitrate influx by turbulent mixing, bottle incubations with nutrient gradients (EXPa) were conducted at 8 stations in November 2016 and 2017. For microzooplankton grazing on phytoplankton, the dilution experiments (EXPb) followed by Landry and Hasset (1982) were done at 8 stations in November 2017 (Fig 1b, Table 1).”

Typically in dilution exp, nutrients were amended in all bottles of the 4 dilution factors. Then, to evaluate whether nutrient limitation exists, additional no nutrient amended exp is conducted for non-diluted bottles (100%). Is this the protocol in the EXPb? Please clarify. If the authors did not follow this protocol, please

explain why.

>Non-diluted bottles without nutrients were made for EXPb due to comparisons of phytoplankton growths between enriched and non-enriched series. Thus, we revised the explanation on dilution experiments like this.

"For evaluating nutrient limitation on phytoplankton growth, no enrichment was conducted for triplicate non-diluted bottles (100%) for EXPb."

Line 100: Please explain how the chla data from different size fraction was obtained in this section.

>Thanks. We described the size fractions as follows.

"Size fractions were defined as Pico for chlorophyll smaller than 2 μm, Nano for chlorophyll between 2 and 11 μm and Micro for chlorophyll larger than 11 μm"

Line 120: Please clarify the difference between the Ct in equation (2) and (3). The explanation is confusing.

>Thanks for comments. We used C't and C'o for EXPb.

Results

Line 131: confidence interval of "what"?

>Thanks. We revised this phrase like this.

"We obtained 16 pairs of vertical profiles for turbulent diffusivity and nitrate concentrations and estimated the averages and 95 percent confidence intervals of the vertical profiles."

Line 136: what is "O"? I cannot understand this sentence.

>"*O*" means "order level". Such descriptions are likely common for physical oceanography.

Line 164: Is the "N concentration" the nitrate concentration in the control groups at the start of incubation, i.e. the nitrate concentration in the ambient seawater without enrichment?

>Yes, we changed "at the start of the incubations" into "in the ambient seawater without enrichment".

Line 179: do you mean "gen'=gmax-m"?

>Yes, we do. We did not change the phrase.

Line 184: Do you mean gen here when referring to net growth rate?

>Yes, we do.

Discussion

Line 191: should be ""previous", not previously

>We revised it. Thanks.

Line 225: This sentence is confusing. Previous sentence said that "microzooplankton standing stocks and growths are not elevated".

>We revised the phrase like this.

"Increase of micro-heterotrophs standing stocks and their trophic transfer to mesozooplankton might be found in the further downstream of the Kuroshio Current."

Line 235: Because microzooplankton growth rate and standing stocks are NOT significantly elevated, I am NOT sure that the authors can conclude the "rapidly transferred to microzooplankton via their grazing".

>Thanks for suggestion. We revised the phrase as follows.

"Our study has provided the first experimental evidence that phytoplankton standing stocks and growths are stimulated by turbulent nutrient fluxes and rapidly grazed by microzooplankton."

Figures:

Figure 2a: The unit of the orange curve seems to be the vertical gradient of nitrate, not the concentration. Please confirm whether this is the concentration or gradient curve.

>Yes, you are right. We revised "nitrate gradient curve" in the caption.

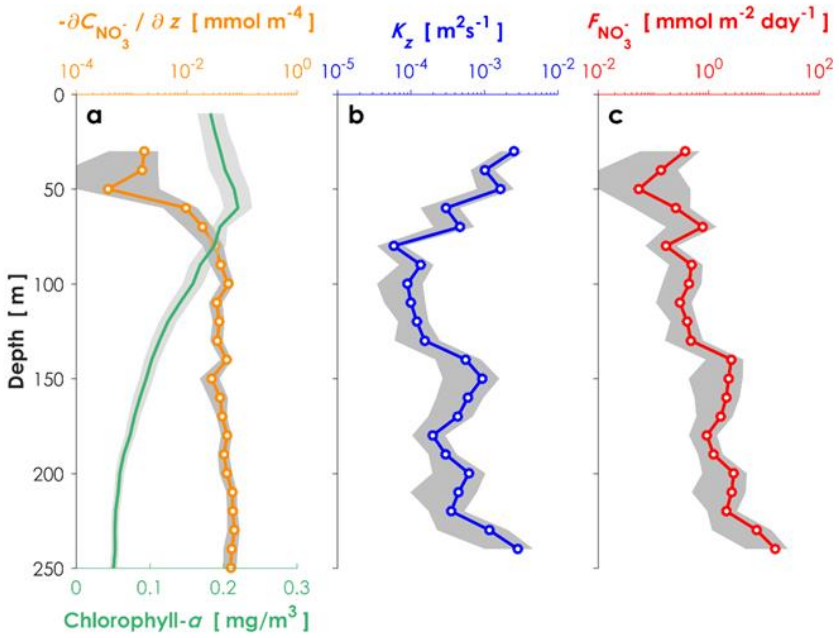

Fig 2. (Kobari et al.)

Figure 3b and 4b: Please use a different set of colors or shading to present the microzooplankton data. It is a little bit difficult to recognize the difference between subplots a and b in these two figures.

>Thanks for comments. We changed the colors.

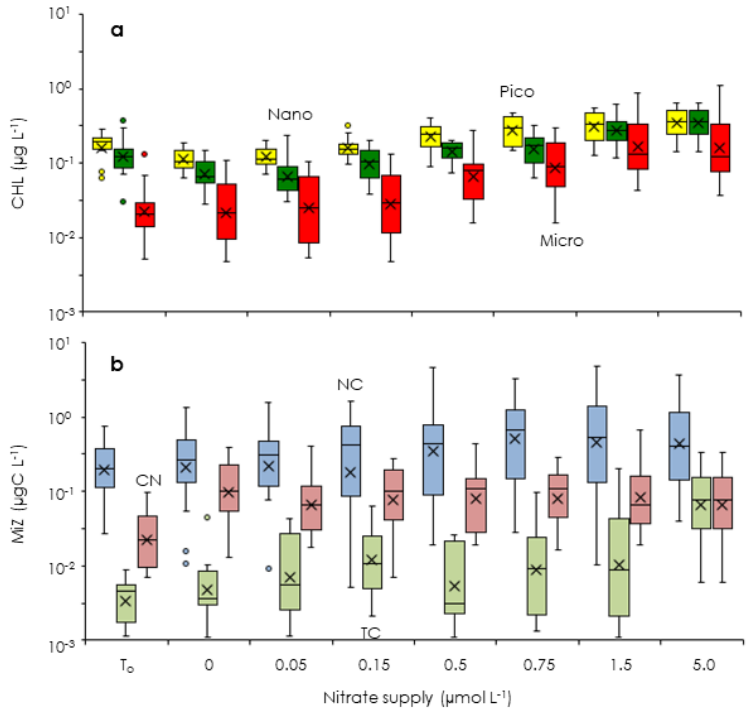

Fig 3. (Kobari et al.)

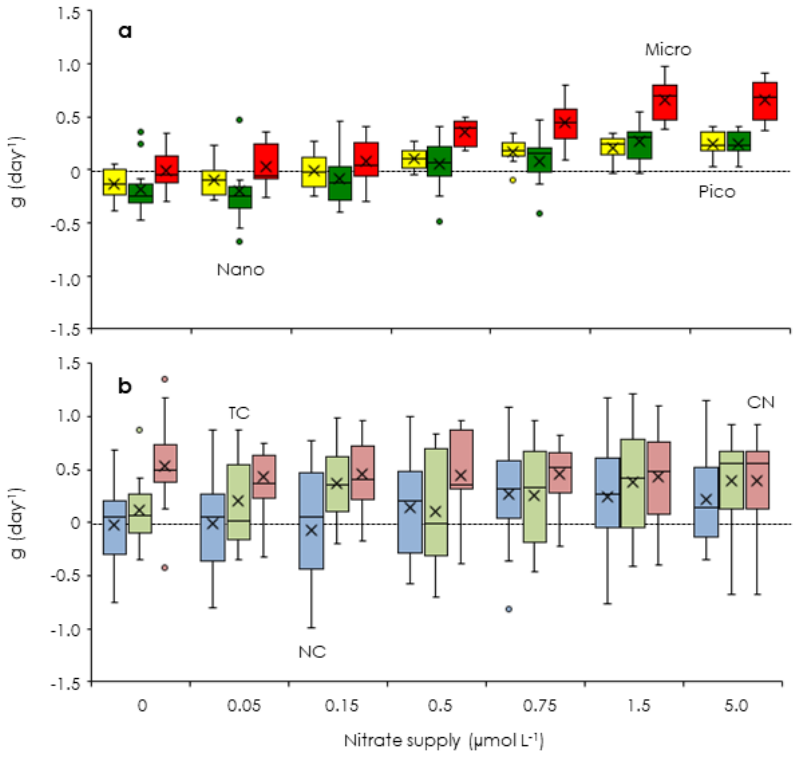

Fig 4. (Kobari et al.)

Figure 5: The color used to present the r values should be consistent to the color used in Figure 3, 4, and 6 (micro = red, nano = green, and pico = yellow). I found that the colors of the points used in this figure correspond to the right size classes but colors of the captions on this figure seem not (micro = green, nano = red, pico = black).

>Thanks too. We used same colors among the figures.

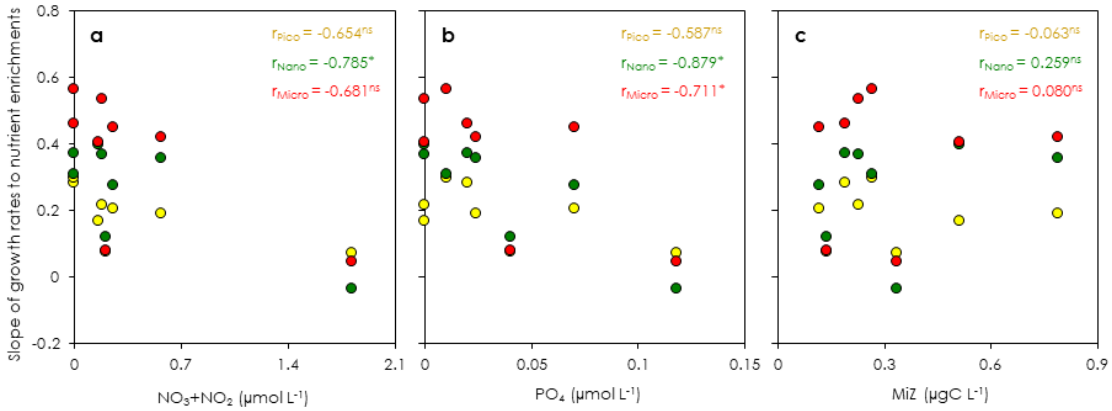

Fig 5. (Kobari et al.)