nature portfolio

Peer Review File

The global distribution and climate resilience of marine heterotrophic prokaryotes

Editorial note: This manuscript has been previously reviewed at another journal that is not operating a transparent peer review scheme. This document only contains reviewer comments and rebuttal letters for versions considered at *Nature Communications*. Mentions of the other journal have been redacted.

REVIEWER COMMENTS

Reviewer #2 (Remarks to the Author):

Review of "Warming-driven shifts in the biomass and respiration of heterotrophic prokaryotes could reduce ocean carbon sequestration"

Authors have synthesized a global data set of heterotrophic prokaryote abundance, cellular carbon content and prokaryotic respiration rates (PRR) and perform a series of statistical analyses illustrating its global distribution and changes with environmental forcing factors. They find that the global variation in heterotrophic prokaryote vary by a factor of roughly two - much less than the several orders of magnitude found for eukaryotes - and increases in prokaryotic biomass and respiration with changes in temperature. They then cast their results in a global change context comparing projected changes in heterotrophic prokaryote abundances with projected changes in higher trophic levels using ESM future scenario output.

I have reviewed this paper previously for [redacted] and now for Nature Communications. I commend the authors for taking the time to improve the article based upon the reviewers' comments before submitted their article to Nature Communications. This does not happen enough in my opinion. I am satisfied with the changes made by the authors on my initial comments on their paper and think that this submission is acceptable for publication in Nature Communications given resolution of the few issues below.

First, I have a high degree of uneasiness with the prokaryotic respiration rate (PRR) determinations and how robust that analysis actually is. As I understand what was done, PRR is calculated as the difference between the specific-production rate (SPR) and the

prokaryotic growth efficiency (PGE). The available data sets for SPR and PGE are not collocated (extended data fig 3). So, each are first GAM-modeled and then PRR is calculated as the difference between the model output. This strikes me as a risky calculation to attempt, especially given the extreme sparseness of the data sets used (especially the PGE data set). I am not a microbiologist, but it is my understanding that there are many assumptions that go into the calculation of SPR and PGE, which will easily get compounded when differences are calculated to arrive upon PRR. It seems that authors need to constrain the uncertainty in the PRR results that could be arising from and measurement and parametric uncertainty and the extreme sparseness of the data sets used. Confidence bounds for the PRR estimates need to be determined before this work is published. As of now, it appears poorly constrained at best.

Last, I object to the title. I know it's hot to talk about "carbon sequestration", but this story is not about carbon sequestration. The paper is about trends in heterotrophic prokaryote biomass and metabolism. I know you have the weaselly words of "all things being equal" at the end of the abstract, but that is insufficient. We are living in a time where things are too often taken out of context and it is our responsibility to make sure we are reporting science and not making up news. Thus, I would retitle this manuscript with a title that is more fitting of the work that you actually performed.

Reviewer #3 (Remarks to the Author):

I thank the reviewers for their thoughtful responses to all three reviewers (I was one of the original reviewers). I have no further concerns or questions.

To both reviewers:
Thank you for reviewing our manuscript again. We are pleased you are satisfied with the changes made in response to your initial comments.

REVIEWER COMMENTS

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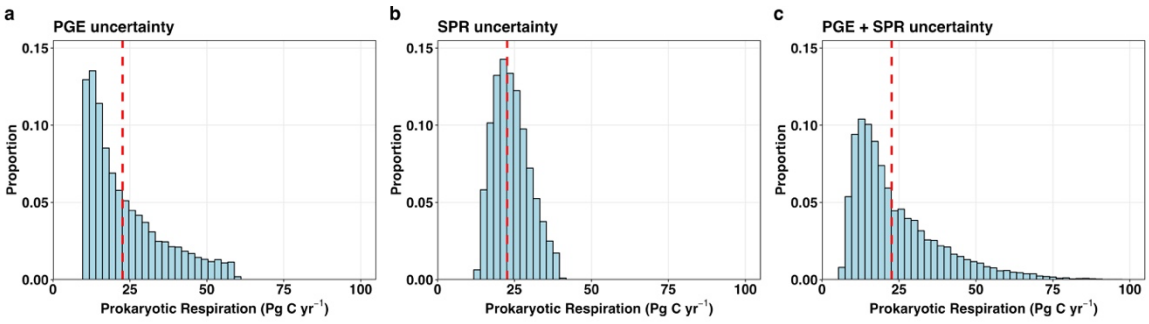
Changed. This is an excellent point, particularly your comment about how uncertainties are compounded when SPR and PGE are used to calculate SRRs. We have added an additional paragraph to the discussion, and an associated extended data figure to

discuss these uncertainties, and quantify the uncertainty in our global PRR estimate (lines 323-342):

We estimate that contemporary global prokaryotic respiration in epipelagic waters is ~22.6 Pg C yr^{-1}. This estimate was obtained using global datasets of prokaryotic specific-production rates (SPRs) and prokaryotic growth efficiency (PGE) which together give specific-respiration rates (SRRs): SRR = SPR/PGE - SPR. There are large uncertainties associated with both SPR and PGE, which are compounded in our estimate of SRR (Extended Data Fig. 9). For example, we used the median PGE from our dataset of 14% to calculate SRRs (Methods), but if we recalculate SRR across the interquartile range of PGE from our dataset (6-27%), the resulting interquartile range of global prokaryotic respiration is ~14-30 Pg C yr^{-1} (with a full range of ~10-60 Pg C yr^{-1}; Extended Data Fig. 9a). Similarly, across the 95% confidence interval for our statistical model of SPRs (Extended Data Fig. 4), the interquartile range of global prokaryotic respiration is ~20-28 Pg C yr^{-1} (with a full range of ~14-40 Pg C yr^{-1}; Extended Data Fig. 9b). When these two sources of uncertainty are combined, the interquartile range of global prokaryotic respiration is ~14-31 Pg C yr^{-1} (with a full range of ~6-101Pg C yr^{-1}; Extended Data Fig. 9c). As shown by these comparisons, PGE uncertainty—likely resulting from the sparseness of available data—was the largest driver of total uncertainty in our estimate. Nevertheless, despite the large uncertainty suggested by the variability of measured PGE, our estimate of ~22.6 Pg C yr^{-1} contemporary global prokaryotic respiration is remarkably similar to two independent assessments: 20.5 Pg C yr^{-1} from a recently published global biogeochemical model[5]; and 20.4 Pg C yr^{-1} using empirical relationships from Lopez-Urrutia et al. (2007)[62] and assuming 10% of prokaryotes are metabolically active[36].



Extended Data Figure 9 | Sources of uncertainty in global epipelagic prokaryotic respiration estimate. *Distribution of 10,000 estimates of global prokaryotic respiration (Pg C yr^{-1}), incorporating uncertainty from a) only prokaryotic growth efficiency (PGE); b) only specific-production rates (SPRs) and c) both PGE and SPR. The red dashed line is our reported estimate of global prokaryotic respiration (22.6 Pg C yr^{-1}). PGE uncertainty was incorporated by sampling from the interquartile range of our PGE dataset (6-38%; assuming a uniform distribution), while SPR uncertainty was resolved by drawing from the SPR statistical model's 95% confidence interval (Extended Data Fig. 4).*

We also now mention PGE uncertainty in another paragraph of the discussion (lines 353-357, addition underlined):

"The wide uncertainties in the temperature sensitivity of prokaryotic respiration—<u>as well as uncertainties in PGE as highlighted above</u>—present significant risks for a growing global population, whose demand on marine ecosystems for food (from fisheries) and carbon storage in the deep ocean will almost certainly increase this century[68–70]."

Last, I object to the title. I know it's hot to talk about "carbon sequestration", but this story is not about carbon sequestration. The paper is about trends in heterotrophic prokaryote biomass and metabolism. I know you have the weaselly words of "all things being equal" at the end of the abstract, but that is insufficient. We are living in a time where things are too often taken out of context and it is our responsibility to make sure we are reporting science and not making up news. Thus, I would retitle this manuscript with a title that is more fitting of the work that you actually performed.

Changed. Thank you for this note of caution. We have changed the title back to the original: *The global distribution and climate resilience of marine heterotrophic prokaryotes,* as we agree that is more reflective of our manuscript's message.

Reviewer #3:

I thank the reviewers for their thoughtful responses to all three reviewers (I was one of the original reviewers). I have no further concerns or questions.

REVIEWERS' COMMENTS

Reviewer #2 (Remarks to the Author):

I have read the authors' responses to my last review queries and am satisfied with their answers.