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Interactive comment on "Reviews and syntheses: Heterotrophic fixation of inorganic carbon – significant but invisible flux in global carbon cycling" *by* Alexander Braun et al.

Alexander Braun et al.

christian.griebler@univie.ac.at

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We thank Rev 1 for the valuable critisism and recommendations.Anonymous Referee #1

Anonymous Referee #1 I was very excited to see this synthesis and review paper on heterotrophic CO2 fixation because heterotrophic CO2 fixation is currently not well understood despite indications that it is quantitatively important in several ecosystems. I started reading the manuscript with great interest, but unfortunately, found the manuscript increasingly disappointing for the following reasons. (1) Simplistic estima-

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tion of global fluxes: The main synthesis work done by the authors in the present study is summarized in the two tables of the manuscript. Table 1 gives the global standing stock of organic carbon in living biomass and the contribution from anaplerotic CO2 fixation. In this table, the authors compiled data on C stocks in different biomass pools. Next, they multiplied the stocks of biomass of heterotrophs by 0.02 and 0.08 and the stocks of biomass of photoautotrophs by 0.01 and 0.05 in order to estimate the contribution from anaplerotic CO2 fixation. Likewise, in Table 2 the authors compiled data on the annual global heterotrophic carbon biomass production for different ecosystems and multiply it by 0.02 and 0.08 in order to estimate the contribution from anaplerotic CO2 fixation. In the first part of the manuscript, the authors point out that there is large uncertainty concerning the rates of microbial heterotrophic CO2 fixation and the underlying metabolic pathways. I agree with this view, and was very surprised to see that the authors estimate global rates of heterotrophic CO2 fixation based on a simple multiplication. I find it highly questionable to base a review paper on this kind of back on the envelop calculation, and I do not see the value of this estimation given how little we know about the size of the flux in different ecosystems.

Reply: We are sorry for the disappointment. After careful reflection and thinking we revised the MS according this point of criticism in the following way. The major focus of the MS is not anymore on the global estimates. We included now a substantial amount of new information including quantitative data from different environments (aquatic systems and soils). See also the new table 1. In the last section of the MS we still present a first global estimation, however, now it is even more conservative (we assume 1-5% of biomass carbon to originate from analplerotic DIC fixation), and the respective table have been moved into the Supplimentary Information. The big uncertainties are repeatedly mentioned in the MS.

(2) Lack of synthesis of empirical data: From a review and synthesis paper on heterotrophic CO2 fixation, I would expect a review of empirically determined CO2 fixation rates. Unfortunately, this is lacking in the present manuscript. For some inspiration, the

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authors should have a look at the recent study by Akinyede et al., 2020 who nicely compiled data on heterotrophic CO2 fixation in soils in a table that gives a good overview.

Reply: Thank you for this valid suggestion. After consultation of the table in Akinyede et al. (2020), which reports 'dark' CO2 fixation rates, we have compiled a synthesis table including all empirical data we could find. Our table compiles only studies where a significant contribution of DIC fixation from heterotrophs is reported.

(3) Unclear scope and unbalanced review of literature: According to the title and the Introduction of the manuscript, the topic of the manuscript is heterotrophic fixation of inorganic carbon. However, the manuscript focuses very strongly on CO2 fixation through anaplerotic reactions and pays less attention to other pathways of heterotrophic CO2 fixation. More importantly, the manuscript concentrates strongly on literature about aquatic ecosystems and largely ignores literature about heterotrophic CO2 fixation in terrestrial ecosystems. This is problematic given that the authors state that the manuscript has a global scope.

Reply: We agree with the reviewer that the MS's content was unbalanced. The strong focus on marine habitats has now been balanced by new information from limnic and terrestrial (soils) ecosystems incorporated, not only in the new Table 1 but also in the text sections. The second point of criticism , i.e. exclusive focus on anaplerosis, is difficult to address. While we have included more information and citations with respect to carboxylases others than involved in anaplerosis, there is no quantitative data on CO2 fixation rates by these other enzymes and pathways. That's why our conservative estimations base exclusively on anaplerosis which is ubiquitously present in all organisms.

(4) Unfocused and unclear figures: I like Fig. 2 because it gives a good overview over different pathways. However, I did not understand the purpose of Fig. 1 and the reason why it only shows one pathway. I guess that the purpose of the figure is mainly to show that the CO2 can be derived either from cell-internal or cell-external processes. While

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this might be important for the estimation of fluxes, I'm not fully convinced that this requires a separate figure. More importantly, I did not understand Fig. 3. According to the caption the figure shows how much CO2 is fixed when microbes feed on different carbon substrates. However, somehow the gist seems to be that this is always 8% as indicated by the grey area. What I did not understand is the meaning of the black line and why it goes up all the way to 45%. Maybe a legend would be helpful here? I am sorry that I cannot provide a more positive review of this synthesis study. I hope that my criticism is clear and that it provides some guidance on how to improve the synthesis study.

Reply: Thank you for this comment. Yes, Fig. 1 was intended to highlight the possibility of a simultaneous fixation of internal and external DIC sources. However, the reviewer is absolutely right, it is too simplistic in ignoring other pathways. We deleted Fig. 1. In Figure 3 (now Fig. 2) we revised the caption text. Now what's seen in the figure should be clear. In detail, der red arrows depict empirical measurements on how much biomass carbon was contributed from DIC fixation. The grey area highlights the general range (1-8%) found in various studies. Right from the dashed line, further carboxylase reactions contribute, beyond the 1-8% range, to the much higher assimilation of DIC. See caption in new Fig. 2.

References Akinyede, R., Taubert, M., Schrumpf, M., Trumbore, S., & Küsel, K. (2020). Rates of dark CO2 fixation are driven by microbial biomass in a temperate forest soil. Soil Biology and Biochemistry, 150, 107950.

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