



# ***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

Received and published: 26 April 2021

We thank Rev 2 for the valuable criticism and recommendations.

Anonymous Referee #2 Broadly speaking, this is a timely review on carbon dioxide fixation by heterotrophs, a process that is likely significant in many biomes, yet typically overlooked in biogeochemical studies. Even if I appreciate that the authors bring up this topic, the manuscript suffers from being too broad in scope and my first and most important recommendation would be to limit the synthesis to the marine environment as this seems to be where most of the relevant cited papers are from. This would

[Printer-friendly version](#)

[Discussion paper](#)

make the manuscript more coherent and informative while also limiting the speculative elements. Specific comments provided below.

Reply: We appreciate this overall comment. It is agreed that based on the original content and structure of our MS, one may derive this conclusion. However, the attempt was to not focus on marine systems. Instead of limiting the synthesis to the marine environment, we took a big effort to balance the information provided from marine studies with information from limnic and terrestrial (soils) ecosystems. The synthesis paper now combines all quantitative data on heterotrophic DIC fixation from aquatic and terrestrial habitats we could find. See new Table 1.

Figure 1 is overall appealing and clear, but what is not evident is where the data supporting the quantitative information comes from. It is stated that the thickness of the arrows represents the relative contribution to fluxes. This is likely context depending and might be relevant in some (but certainly not all) biological systems and it needs to be clearly specified (and referenced) how this quantitative information was obtained and under what circumstances they are valid. This will most likely also vary between different heterotrophs and this need to come across in the figure.

Reply: Thank you for this comment. We realized that Fig. 1 while being appealing is too simplistic and rather represents a certain situation/organisms than a general pattern. Because of similar comments received from Rev 1, we decided to remove Fig 1. To our opinion, it is not essential and all information is somehow provided also in the text.

I have similar concerns for figure 3, as it is not evident whether the (again) quantitative information derive from purely theoretical reasoning or if there is empirical evidence? In the latter case I of course want to know under what conditions these results are valid and how they were obtained. References needed! In line with these comments, I found that a lot of the inferences and assumptions made for natural communities are based on early work with pure cultures. It is not evident that such extrapolations are valid. A more critical discussion about this is needed. One fundament of the

arguments made is the strong link between the degree of reduction of the substrate being metabolized in relation to the same metric for (average) biomass. I think this is an interesting and potentially very useful approach, but biomass is not only carbon. Also other elements (nitrogen, sulfur, etc) are assimilated in large quantities and these can also have different degree of reduction (ammonia and nitrate as an example). This can of course also have a major impact on the need for anaplerotic CO<sub>2</sub> fixation but is completely overlooked in this synthesis.

Reply: The comments are appreciated. First of all, we revised the caption of the former Fig. 3, which is Figure 2 now. A much better and clearer explanation is provided. The figure contains real data (e.g. the red arrows depicting at the fraction of biomass carbon contributed from DIC fixation in different microbes when growing on different organic carbon sources) and in the new text added, the aspect of additional DIC fixation along with the oxidation of highly reduced organic carbon sources was elaborated more extensively. Several new references have been added. It is true that also other elements with species of differing degree of reduction (e.g. ammonia and nitrate) are assimilated. However, the overall amount is general one order of magnitude less. We do not think that this will interfere with the DIC fixation patterns by heterotrophs introduced here. In case of chemoautotrophy (e.g. DIC fixation in combination of ammonia oxidation by nitrifiers) of course, the amount of DIC fixed does play a role in biomass production. This is already mentioned at several spots in the MS.

I strongly object to the simplistic way of estimating the quantitative significance of heterotrophic CO<sub>2</sub> fixation as presented in tables 1-2. This is way too speculative. This type of estimates should not be extrapolated beyond the environments where the process has actually been quantified with reliable modern methods. Assuming an equal percentage of heterotroph biomass to come from inorganic carbon fixation will most certainly be misleading, especially moving from the reasonably well studied oceanic waters to terrestrial and deep biosphere biomes where very different conditions may prevail. I would strongly encourage the authors to show some restraint here. One so-

lution could (again) be to change the stated scope of the review to focus on marine waters where it might be at least somewhat reasonable to make such assumptions.

A similar comment was received by Rev 1. Thank you for addressing this critical issue. As already mentioned above, we have changed the following. First, we recalculated our global estimates with 1-5% of heterotrophically fixed DIC contributing to biomass carbon. This leads to a more conservative estimation. All studies conducted, as well as theoretical calculations, underline that a minimum of 1% can be expected for all kind of organisms independent of the habitat they live in, and 5% is still very reasonable and can be understood of an upper value. However, to move the global estimates and the two tables out of the focus, we provide them now as Supplementary Information, and the discussion on global carbon stock and assimilation rates from anaplerosis is only a brief text section at the end of the MS. Moreover we included a pile of data on quantitative estimates from individual habitats intending a balance between information from aquatic and terrestrial systems; see new Table 1.

Reply: The cited papers include both modern and old cited papers (some from the 1960's) and I doubt methods and approaches for quantifying and documenting heterotrophic carbon fixation have remained the same. Methodological constraints and biases may play a major role here as the typically rather slow anaplerotic carbon fixation and other heterotrophic bacterial CO<sub>2</sub> fixation can be easily masked (or “contaminated”) by other metabolic processes. Some critical review and account of the different methodological approaches used in the cited work would have been very valuable and useful for the reader. Are results from the 1960's more or less trustworthy that results obtained 50-60 years later? Thank you for this comment. We actually have thought of this aspect when putting the information together. Our impression is that most of the early studies, done by biochemists, microbiologists and physiologists, deliver reliable information, with the weakness that data come from only laboratory experiments with microbial strains and tissue cultures. With some of the modern ecological studies, it is less clear what have been measured, as dark CO<sub>2</sub> fixation includes both chemoau-

totrophs and heterotrophs as actors. Honestly, we think that this issue is not really of relevance, because the numbers on heterotrophic CO<sub>2</sub> fixation as contribution to biomass carbon are always in the same range (about 1-8%, with some higher values too), independent from the time of the experiments or studies. In other words, we did not detect a systematic change in values from older studies to modern ones. We conclude that a separate discussion about this aspect is thus not necessary.

I find the arguments about carbon use efficiency confusing (line 133-150). While the issues with using oxygen removal to deduce respiratory changes in carbon dioxide is quite well known and recognized in literature, but this may surely lead to both overestimates and underestimates of CO<sub>2</sub> production depending on the particular context. With that in mind it is not clear to me how this leads the authors to the following conclusion: “Collectively, with respect to C cycling, heterotrophic CO<sub>2</sub> fixation and the carbon flux from the inorganic pool into heterotrophic biomass can be regarded as a process more important than hitherto assumed”. This may not be true for all marine conditions and certainly not for systems such as the deep biosphere, soils, freshwaters and other systems where other constraints may prevail and where the methodological tradition differ.

Reply: Sorry that our line of argumentation was not clear. What we wanted to say is that frequently the amount of CO<sub>2</sub> produced by oxidation when being calculated via the amount of O<sub>2</sub> consumed, does not take the ‘recycling’ of CO<sub>2</sub> via anaplerosis into account. Moreover, the contribution of anaplerosis and other carboxylation reaction not linearly correlate with O<sub>2</sub> consumption. I hope we have made this now clear in the revised text. As done in Akinyede et al. 2020, one could calculate the CO<sub>2</sub> not emitted from a habitat due to heterotrophic assimilation. Strict measurements of O<sub>2</sub> consumption (common in aquatic environments) and respiration (common in soil environments) do not provide this information.

Some additional specific comments: Line 42: Actually, these rates have been quantified in numerous studies in various ecosystems and for various organisms. Please

revise.

Reply: Yes, true. This is exactly what we show with our MS. We changed this sentence accordingly.

Line 94-95: An alternative for adjusting the degree of reduction of the substrate to that of average biomass is of course to oxidize the organic substrates in respiratory processes and at the same time gain energy for cellular processes and assembly. This is quite evident and the authors also make this clear elsewhere in the text. Nevertheless, the current statement made here needs to be adjusted.

Reply: We are sorry but we did not fully understand this comment. What we say is: If the organic matter that is oxidized in respiratory processes (aerobic and anaerobic) is more reduced than the organisms' biomass carbon further CO<sub>2</sub> is fixed in addition to what is fixed already in anaerobic reactions. As an extreme example methanotrophy is mentioned. Energy gained by the catabolic reaction (respiration process) is used to build biomass. A special case is spilling reactions where organic carbon is in excess but growth is limited by essential nutrients. We have mentioned that in the MS. We hope, we are clear now.

Line 153-155: Confusing statement as chemolithoautotrophs which dominate in many dark environments are also autotrophs. Again: focus on marine systems and by making some more specific statements for this biome, the story will be more substantiated and to the point.

Reply: The sentence was corrected to avoid confusion.

Line 152-207: There is a quite substantial body on literature about this, but here the discussion is more or less exclusively about work in the oceans. See earlier comments about this bias in referenced literature.

Reply: We agree. We have now implemented new information on limnic systems and terrestrial environments to balance the synthesis. A focus on marine systems was

never intended.

Line 215-218: To make this assumption beyond oceans you would also need to present data demonstrating that the 2-8% is also valid for the other biomes.

Reply: This is a valid comment. We have now incorporated data from all different kinds of organisms and different biomes to better support our assumptions.

Line 234: “scares” should be “scarce”

Reply: Changed accordingly.

Line 340: is “exemplarily” really the right word here?

Reply: Changed accordingly.

Line 353: Why introduce the abbreviation? Is it used anywhere else?

Reply: Abbreviation deleted.

Line 353-360: This is very speculative and relies on the assumption that heterotrophic CO<sub>2</sub> fixation is and will remain a constant proportion of heterotrophic production. Where is the evidence for that? I do not find this likely given the current information we have about the metabolic diversity of microbial communities. The argument about methanotrophs is more credible as they evidently use much more inorganic carbon in their anabolic processes.

Reply: We think, a minimum of DIC is fixed by every heterotrophic organism. This allows a first estimation. We agree that at certain physiological and environmental conditions it will be more, but never less than 1% of biomass carbon which is our minimum assumption. Evidence comes from numerous experiments with microbial strains, tissue cultures, metazoan, and also from a number of field studies, including soils, drinking water biofilms, activated sludge, to give some examples. We accordingly revised the text to include this information.

[Printer-friendly version](#)[Discussion paper](#)

Printer-friendly version

Discussion paper