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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.

Anonymous Referee #2

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

Figure 1 is overall appealing and clear, but what is not evident is where the data sup-

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porting 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.

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 CO2 fixation but is completely overlooked in this synthesis.

I strongly object to the simplistic way of estimating the quantitative significance of heterotrophic CO2 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 solution 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.

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 CO2 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?

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 CO2 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 CO2 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 constrains may prevail and where the methodological tradition differ.

Some additional specific comments:

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

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.

Line 153-155: Confusing statement as chemolitoautotrophs 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

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.

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.

Line 234: “scares” should be “scarce”

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

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

Line 353-360: This is very speculative and relies on the assumption that heterotrophic CO2 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.

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