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Interactive comment on "Temperature-mediated changes in microbial carbon use efficiency and ^{13}C discrimination" *by* C. A. Lehmeier et al.

Anonymous Referee #3

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This manuscript reports on results from a series of chemostat-based (at steady state) measurements of respiration and d13C of CO2 from cultures of P. fluorescens at various temperatures. The objective is to study the temperature dependence of microbial CUE and C isotope discrimination. It is clear that solid measurements of CUE are very difficult and thus the understanding of the factors that influence it. It is also clear that the microbial fractionation of C isotopes is a big unanswered question that keeps us from being able to use it to help us understand the C cycle. I applaud the effort by the investigators to try to tackle these very difficult questions and think that there is promise in the approach. However, I see a fair number of aspects that add too much uncertainty to the findings and their interpretations.

The points I consider more critical are explained below and are followed by other sec-

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ondary but also important issues. SGR, SRR, CUE data: CUE is defined as the fraction of SGR out of the sum of SGR and SRR. By definition, the reactor dilution rate is the SGR . (There is not a reference to support this approach). The steady state of the culture is maintained by maintaining the SGR. Because SGR is maintained constant, CUE will always be explained by changes in the SRR (CO2 rate per unit of microbial biomass). Is it really possible to independently assess effects of temperature on specific growth rates, when they will be inevitably determined by the respiration rate and the microbial biomass? Thus is it really possible to estimate CUE? The authors state that "The 50% reduction in steady state dry microbial biomass with increasing temperature was due to 2.5 fold increase in SRR". In my view, given the nature of the thermostat system, the result is rather that SRR increased with temperature due to the decrease in microbial biomass with temperature. There's a circularity that complicates the interpretation of these variables when combined. In my view, the relationship of SRR and microbial biomass with temperature can be explored with more confidence than CUE and it is valuable that it was done at steady state.

The extent of the impact of secretion/waste on the estimation of uptake (SGR+SRR) is difficult to constrain realistically and also its variation with temperature without any measurement of what was actually in the solution after filtration. How about the contribution of the further uptake and respiration of those substrates? (on a somewhat related note: the lack of enzymatic activity in the solution may mean that the enzymes are being quickly uptaken and thus are not detectable; the current interpretation of the lack of enzymes is very speculative).

Because of these uncertainties the overall interpretations and general discussion on the effect of temperature on CUE are challenging to make.

Isotopic discrimination data: in my view there is too much uncertainty in what happened with the C during the experiments and this is combined with various unexpected hard- to- explain observations. A full budget approach, accounting for all pools (both their size and isotopic composition), including inorganic C, dissolved organic (not cel-

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lobiose) could have potentially allowed explanations to allow confident interpretations. The uncertainty in the potential reasons for the very d13C values in the early incubations, and more importantly the reasons for the gradual change towards 'real' values is a problem. We don't know to what extent the processes at play during the 'climbing' phase are still at play during the plateau. The carbonate system explanation for the early stages would need measurement of the pool of inorganic C.

The observation that microbial biomass was depleted in 13C relative to the substrate is surprising given findings of previous much simpler studies. If they are depleted we would expect enrichment of the respiration, which what not the case. What accounts for the further depletion of the respiration is too hard to explain and it is seriously speculative. It is surprising that the d13C values of the filtrate are not presented and that an attempt to partition is not done. They suggest microbial discrimination against heavy (enriched) substrate. With a d13C value of -24, the atom percent of 13C is very low. Could the actual amount of potential 13C to discriminate against explain the actual degree of enrichment? Again, a budget approach would have helped here.

The observation of strong fluctuations in the microbial and respiration with temperature is very (very) hard to explain and grasp and the current attempt is highly speculative. I also wonder what would have happened if the runs had been replicated and the contribution of experimental error to the ups and downs.

The combined uncertainties in the isotope data and metabolism data then make the discussion on the relationship between them a bit of a stretch.

Introduction -More background on the connection between metabolism and isotopic discrimination would be nice to have. -There's not sufficient background on the factors that may drive microbial C discrimination. Methods -what is the material of the filters? Could filter aDsorb some molecules that the " removal" of material from the filter would not get?

Interactive comment on Biogeosciences Discuss., 12, 17367, 2015.