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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.

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General comments: "Temperature-mediated changes in microbial carbon use efficiency and 13C discrimination" by Lehmeier and co-authors is a well-written manuscript that addresses questions of interest to a wide range of BG readers. The authors used stable isotope tracers and a flow-through chemostat with a single species and single carbon substrate to identify temperature controls on microbial carbon use efficiency as well as the discrimination against 13C during respiration. This study offers unique insights into the role of temperature for microbial carbon cycling and contributions to 13C-CO2 signatures.

In fact, while the authors focus on broader applications for soil and terrestrial C cycling,

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these results are also extremely relevant for freshwater and marine biogeochemistry and microbial ecology. Perhaps even moreso given the chemostat conditions, which may be more appropriately applied to aquatic ecosystems. The authors could reach a broader audience by acknowledging this in the language, scope, and citations of the introduction/discussion (sometimes just a matter of deleting “soil”).

Response: Thank you to the reviewer for highlighting that the insights our approach reveals are simultaneously unique yet applicable to diverse systems. We agree with the reviewer that the relevance of our work for other disciplines should be acknowledged more in the manuscript. We will provide context from other disciplines in the introduction, and weave this idea into our discussion as well. We have selected multiple citations useful for these changes (e.g. Goldman & Dennet, 2000; Pomeroy & Wiebe, 2001; Chrzanowski & Grover, 2008; Hall et al., 2008).

The chemostat set-up and equilibrium assumptions are very clearly described. I do believe the authors could be more up-front about the unknowns associated with equal labeling of cellulose and glucose within the labeled cellobiose substrate, and what this might mean for the interpretation of the discrimination results (there are hints of this in EEA methods and results/discussion, but this seems to be an unknown with significant consequences for results).

Response: The δ13C value of repeated measurements of the cellobiose substrate was -24.2‰ with little variation ($\pm$0.04 1SE, n=12, Fig. 4), showing that the substrate was a homogeneous mixture. (at one stage in the manuscript, we wrote -22.4‰ for cellobiose δ13C; this was a typo that will be changed.) Measured against VPDB standard, the δ13C of -24.2‰ implies a 13C/12C ratio in the sample of $\sim$1/91. Considering the molecular formula of cellobiose C12H22O11, this means that not more than about one out of eight cellobiose molecules in the supplied substrate had a 13C atom incorporated. We have not determined at which positions a cellobiose molecule may have had that 13C atom incorporated. We have, however, confirmation from the supplier (Sigma-Aldrich) that the cellobiose we used was of biogenic origin. Evidence from

BGD
12, C10277–C10282, 2016

work on carbon isotope distribution within carbohydrate molecules (e.g., Rossmann et al., 1991; Gleixner & Schmidt, 1997; and references therein) suggests non-random distribution of 13C in biological molecules, probably mainly caused by kinetic isotope effects in enzymatic reactions (see also Tcherkez at al., 2012). Based on this phenomenon, the 13C atom in a 13C-containing cellobiose molecule we supplied to the microbes will not be randomly distributed within the molecule but rather consistently on the same position. In our view, this rules out the possibility that any changes in the 13C distribution within the cellobiose substrate supplied to the microbes were responsible for the δ13C patterns in biomass and respired CO2 we observed (Fig. 4).

There are still unknowns in isotope effects during glycolysis and respiration (Tcherkez et al., 2012), but considering the literature that certain enzymatic reactions in metabolic pathways discriminate against 13C in organic molecules in a predictable way, we believe that our discussion offers parsimonious explanations for the observed isotope patterns in biomass and respired CO2. In a revision, we will include these considerations.

Are there recommendations for how these results can be applied to non-steady state scenarios in heterogeneous soil or biofilm matrices? This study is novel and useful, but drawing connections from steady-state chemostat measurements to the real world remains a challenge.

Response: We agree with the reviewer in that it would be very interesting to have more information about how microbial C and C isotope fluxes during the transformation of organic matter change with environmental/growth conditions.

In our experiments, maintaining microbial growth in steady-state was critical for permitting direct measurement of respiration rates and the δ13C of respired CO2, without the confounding influences that measurements in less controlled environments entail. The steady-state growth was hence a means to obtain data that would otherwise be only very difficult if not impossible to obtain, and to study the effect of temperature on

BGD
12, C10277–C10282, 2016

microbial C and C isotope fluxes in “isolation.” That is, we strived to maintain all other environmental factors constant except temperature.

Microbial experiments in controlled environments always reveal only a snapshot of specific growth and environmental conditions, and accurate generalizations therefore are difficult to make. However, we have no reason to assume that the principles and mechanisms about the temperature response of C and C isotope fluxes identified in our work wouldn’t happen in the “real world” (like, a heterogeneous soil or an aquatic environment). Because our work used a simplified system to explore fundamental microbial respiration rates and respiratory C isotope discrimination during organic matter transformations with varying temperature, it serves as a starting point from which we can formulate new hypotheses, and explore, for instance, the reason for the presumably strong discrimination against 13C-containing cellobiose molecules in the supplied substrate.

Specific comments: Page 17372, line 15 - Is a 1:1 respiratory quotient appropriate for both cellulose and glucose? I would guess cellulose RQ > 1, while glucose RQ_ 1.

Response: From our data we obtained and discussed some circumstantial evidence that Cellobiose was cleaved intercellularly, and that the cleavage products, two Glucose units, then could serve as substrate for respiration. Considering that the group of carbohydrates is generally ascribed an RQ of 1, we used an RQ of 1 at that point of the manuscript to highlight that the supply rate of O2 to the reactor should have exceeded the consumption of O2 by the microorganisms by far, and thus allow for aerobic metabolism of the population in the reactor. If cellobiose had an RQ > 1, then the molar amount of O2 consumed would be lower than the molar amount of CO2 produced, which should support the point of aerobic metabolism in our chemostat reactors even more.

Figures –What is the uncertainty of the results presented in Figures 3, 4, 6?

Response: The data presented in these figures are from seven independent chemo-

BGD

12, C10277–C10282, 2016

stat runs, conducted at seven different temperatures. Therefore, we were unable to provide error bars that would indicate any variation across true experimental replicates. However, in a revision, we could provide measurement uncertainties for the concentration and δ13C of respired CO2, based on large numbers of measurements of reactor headspace CO2 concentrations and δ13C at steady-state.

(please see also below our response to the referee #3 critique to the lack of replicates.)

Figure 5 could more clearly identify the knowns/unknowns beyond boxed and unboxed. Perhaps two panels to show the difference in (a) steady-state chemostat versus (b) soil measurements and the unknowns/challenges for moving forward?

Response: In a revision, we will modify Figure 5 in response to this comment so that differences between steady-state chemostat vs. soil measurements and the unknowns/challenges for moving forward become clearer.

Cited literature:

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BGD

12, C10277–C10282, 2016

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