The University of Kansas

26 February 2015

Dr. Karolien Denef
Associate Editor for Soil
Colorado State University
Ft. Collins, CO, USA

Dear Dr. Denef,

We have submitted a revised version of our manuscript "Investigating microbial transformations of soil organic matter: Synthesizing knowledge from disparate fields to guide new experimentation" by S.A. Billings, L.K. Tiemann, F. Ballantyne IV, C. Lehmeier, and K. Min. Both reviewers provided valuable input that resulted in an improved manuscript. We have responded in full to both reviewers' comments, and detail our responses below. In addition to these specific responses, we cut extraneous text in multiple places throughout the manuscript to reduce its length.

Thank you for your time and consideration.

Sincerely,

Sharon Billings

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Rev #1:
Page 1104, Line 6: Add literature references for ": : :as has been elucidated in the ocean." *Reference has been added.*

Page 1112, Lines 22-26: This sentence is a bit long winded and should be made more concise or split up into 2 sentences. The way it is now, it is difficult to read. *The sentence has been cleaved and reworded for clarity.*

Page 1114, Lines 2-6: Is there a particular reference for this that would help explain the response you are describing? *There is no citation for this concept, as we are hypothesizing about how changing temperature may influence microbial communities in this section. However, we added a sentence after these lines to indicate that this remains unknown.*

Page 1115, Lines 4-5: "In contrast, apparent Ea for the NAGase/NAG reaction appears consistently higher than the corresponding intrinsic Ea." This statement is only based off of a couple of data points in Figure 1. Actually, only at one pH do you have both intrinsic and apparent values that you can compare. Can you add more data to the

graph to support this statement? If not, using 'consistently' in this statement is too bold. Additionally, are their error bars associated with this data? Is this the average values? Are these conclusions based on any statistics? *We agree that this statement was too bold. We are unaware of other studies reporting the apparent Ea of NAGase/NAG reactions in soils, so we cannot further populate the graph, nor can we achieve a balanced data set on which to perform the statistics that might permit us to make stronger statements. We have reworded the lines surrounding this statement in a more conservative manner, added a reference to the section, and have added 95% confidence intervals for the points in the figure representing our own work to enhance the reader's ability to compare intrinsic (our own data) vs. apparent (others' data) activation energies.*

Section 4: You may find this newer publication helpful and worth citing in this section: Tang, J. and W. J. Riley. 2015. Weaker soil carbon-climate feedbacks resulting from microbial and abiotic interactions. Nature Clim. Change 5:56-60. *This is an excellent reference that was published online after we submitted this review. We have added it to our citations in this section.*

Page 1122, Lines 25-28: Add citations. *We have linked a short phrase to the cited sentence that comes after these lines to indicate that these citations are intended for the concept here.*

Figures:
Figure 1: It seems like there would be more apparent NAG Ea values in the literature to add to this graph? *Please see response to the NAGase/NAG comment above.*

Figure 2: Text is hard to read on this figure. May be helpful for the reader to see arrows included in the figure that indicate changes in E_a and mean residence time with depth. *We have improved the clarity of the font of the text within the shapes, but we have not included arrows for E_a or MRT. First, we believe that it's difficult to make generalizations about E_a and MRT of individual compounds as we move through the soil profile, and literature reports conflicting data about the temperature sensitivity of deep SOM during incubations. Therefore, we only indicated a "younger mean age" and a "greater mean age" of material with depth in the left-hand shape of the figure and then further address the MRT issue in the figure legend.*

Rev #2:
Page 1099, lines 4-8: This sentence sounds way too similar to the first sentence of the abstract. I suggest changing one or the other. *We have modified the first lines of the abstract to address this issue.*

Page 1100, lines 8-16: This is all one sentence, and is very long and confusing. Also, maybe backup and summarize the C quality hypothesis? *We have cut this long sentence into multiple sentences, and offer a brief explanation of the CQH.*

Section 2, pages 1102-3: If I read this correctly, you suggest that natural aquatic and chemostat environments help to identify baseline microbe-substrate relationships that may be present in soil. This is mostly due to relief of diffusional constraints. But what if diffusional constraints are the defining characteristic shaping microbe-substrate interactions in soil? I think this may be an important point that, even if addressed later on, should probably be introduced here as well. You could also be more clear about what you mean by 'varying environment' and 'changing conditions'. *We have added a few lines to address this issue:*

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"By turning to natural and artificial aquatic systems for guidance, we do not mean to imply that diffusional constraints are not important. Indeed, they may be the dominant feature driving SOM decay in some intact profiles. However, by exploring these aquatic systems we gain insight to enzymatic and microbial responses to changing environmental conditions in relative isolation from such constraints, and that in turn allows us to assess each factor's relative importance." We would like to retain the use of the generic phrases 'varying environment' and 'changing conditions' to permit flexibility in the use of the concepts we present, but we offer some examples in parentheses of what these phrases can indicate.

Page 1105, lines 21-24: The last sentence of this paragraph seems like the main point of this whole section. Might want to move it up closer to the beginning? *We agree that this would make a nice beginning to the section, but if we move it closer to the section's start, we have to add other phrases to segue into the first paragraph. In an effort to respond to this reviewer's other comments about the need to reduce manuscript length, we'd like to keep this sentence placed as is.*

Page 1116, lines 19-23: Might to add Manzoni et al. 2014 in this sentence? It's already in the reference list. *We have made this insertion.*

Page 1119, last paragraph: Kind of lost me here. Can this be stated more simply as averaging over space or time? Given that you can identify the critical thresholds over which each, or both, vary?

We rewrote a significant portion of this paragraph in an effort to clarify the details of and the need for averaging over space and depth profiles. We are making the point, hopefully now better than before, that to translate what we can learn from chemostat and reductionist approaches, we need a way to place physiological responses occurring at the level of cells in the context of the physical-chemical variability that exists in soils without overwhelming ourselves with complexity. Coarse graining, which can be achieved by multiple methods, provides a way to acknowledge all the fine scale heterogeneity that exists in complicated environments like soils, and allow only the most influential components of heterogeneity to govern SOM transformations and soil respiration at spatial scales relevant for ecosystem modeling.

I may have missed it, but do you discuss what kinds of stresses can be imposed using chemostats? I recall something about osmotic stress earlier. It's often by observing systems under stress that we learn the most about their function. *Yes, in section 2.4 we specifically mention two physiological stressors, rapidly changing matric potential or temperature, which can be isolated and studied. We don't specifically use the word 'stress' earlier, but we also discuss another potential stressor that is manipulated in chemostat studies – nutrient constraints.*

Also, chemostats seem good for interrogating equilibrium systems, but what about disequilibrium conditions? Any options there? *At the beginning of Section 2.2, we have added a line stating that we cannot know microbial growth rates in non-equilibrium conditions; though unfortunate, the benefits of exploring equilibrium conditions are great.*

I found section 5 to be fascinating and very informative. However, this is the section I'd single out for significant reductions in length. The focus seems to drift here, away from the main points of the paper which are: utility of using aquatic and chemostat systems to understand SOM dynamics. *We have tightened this section to make it more explicitly about using the works from aquatic and theoretical realms discussed above to investigate the mysteries of changing SOM properties with depth. In so doing, we shortened the section considerably.*

Higuchi Hall • 2101 Constant Ave., Room 157 • Lawrence, KS 66047-3759
(785) 864-1560 • Fax: (785) 864-1534 • sharon.billings@ku.edu