Lukas Kohl (Referee #2) (Received and published: 16 July 2019)

General comments

Keenan and co-authors investigated the effect of carrion decomposition on the underlying soil. In particular, they studied the spatial extent to a beaver carrion decomposition hotspot changed soil biogeochemical parameters (mainly C:N and d15N) one year post deposition. They find that elevated d15N values due to N inputs from the decomposing beaver were detected to 60cm lateral and 10cm depth.

The manuscript covers an important and understudied topic of terrestrial ecosystem ecology. The authors used state of the art methods and their results justify their conclusions. The manuscript reads very nicely and is surely of high interest to the Biogeosciences readership.

Specific comments

1) I think the main weakness of the manuscript is that the authors pooled all control samples (soils collected in some distance from the placed beavers) and analysed only a single composite sample. This means we cannot know the spatial variability of control soil properties, or the uncertainties associated with the measured average.

> **Response**: We agree that pooling the control soils (a total of 5 independent locations) represents a limitation. Based on our previous studies (e.g., Cobaugh et al., 2015), we knew that the spatial and temporal variability in hotspots is far greater than that what we see in background soils. Therefore, for this experiment we collected several discrete control samples at the beginning of the experiment ("Initial" in Table 1) to assess spatial variability at the site, then a composite control sample at each time point to assess temporal variability. So, while we do not have spatial variability for each time point, we felt this combination approach was sufficient to identify the contrast between background and hotspot processes, which was the overall goal of the study.

2) The manuscript's use of biogeochemistry is somewhat confusing (e.g. L19-21). In my opinion, changes in soil d15N values may result from either changes in soil N biochemistry, or from changes in the d15N values of N inputs to soils. The manuscript's data largely suggest the latter is the dominant effect observed here. Where actual changes in the soil biogeochemistry are implied (again, e.g. L19-21), it would be better to be more specific and describe the changes in soil biogeochemistry that they think are indicated by changes.

> **Response**: If we're interpreting the reviewer's comment correct, it seems they are suggesting that soil δ^{15}N values are driven by either changes to N biogeochemistry ***or*** N inputs. However, there is scientific evidence from other systems that show that it can be combination of both – both inputs and biogeochemical process are contributing. In decomposition hotspots in particular, we know from past research that both of these processes are occurring simultaneously. The nutrient-rich carcass inputs result in enhanced microbial activity (respiration, enzyme activities, N cycling processes, etc.) and shifts in microbial communities, which have been reported in numerous studies (e.g., Macdonald et al. 2014; Cobaugh et al., 2015; Metcalf et al. 2016; Keenan et al., 2018a; Singh et al., 2018). We also directly observed elevated rates of nitrification during this decomposition study (Table 1), which suggests the N input from carcasses stimulates a microbial N cycling response. Because we measured whole system response, we cannot directly link a specific process to an enrichment effect. However, given the strong

evidence for enhanced microbial activities in this system, we have elected to retain our original explanation for the observed results: that the change in soil $\delta^{15}N$ is driven by the carcass inputs in combination with multiple biogeochemical processes.

3) I think that assumptions that are needed for the 13C/15N three-endmember mixing model to calculate input sources for deeper soil layers are likely not met. Such a model assumes that C and N of a given soil sample originate in the same proportions from the same sources, which is not true.

Response: We appreciate the reviewer's thoughtful comments and agree that the assumptions of the model cannot really be met for this system. Therefore have elected to remove the three end-member mixing model from the manuscript. We initially included the model as a way to simplify the system, recognizing that in reality, as the reviewer states, this is a big assumption. Since this model was being used for simplification/illustrative purposes, removing it from the manuscript does not alter the main findings of the study.

4) Furthermore, the authors need to clarify what the mixing model actually estimates (e.g. L223: ".. evaluated the proportional contributions of three distinct sources to the stable isotopic composition in hotspot deep profiles ..") - mixing models do not estimate contributions to the isotopic composition, but to the contribution of distinct sources to a particular pool of matter (soil organic matter, soil nitrogen, etc).

Response: The mixing model used (and subsequently removed in the revised MS) was originally designed to evaluate the proportional contribution of different end members (dietary sources) to a final isotopic composition (animal tissues or the "pool" of organic matter). However, we recognize the limitations of applying this trophic ecology approach towards distinguishing inputs to soil stable isotopic composition, and have removed it from the manuscript.

5) If I understand correctly, I think the authors use this mixing model to distinguish differences in d15N due to depth from differences due to source (soil N vs. beaver N). 13C is used as an additional variable to allow for a third endmember. However, this doesn't work for several reasons. Most importantly, C and N in the same soil sample can have different sources. As a consequence of this, 13C and 15N do not necessarily show linear co-variance through the soil profile. Furthermore, it is not clear if the 15N signature of N inputs is modified as N migrates down along the soil profile. However, I don't think this mixing model is required to support the authors conclusions and I would remove it.

Response: We completely agree with the reviewer and appreciate the suggestion to remove the three end-member mixing model from the MS. We agree that our results and conclusions are still supported by doing so.

6) Similarly, I find the Δ15N values confusing and I'm not sure what they contribute to the manuscripts story. In my opinion, Fig 5a should be sufficient for report that – unlike in control soils– d15N values decrease with depth at the hotspot, representing the recent 15N-enriched N inputs from the top of the soil profile.

Response: We included the $\Delta^{15}N$ values as an additional way to quantify (or characterize) N changes with depth in the soil profile (lines 240-242). This approach

(subtracting soil at depth from the surface layer) calculates the ^{15}N enrichment at each depth relative to the surface and has been used previously to identify soil profiles with perturbed N cycling or disturbed systems (e.g., Hobbie and Ouimette, 2009). These data emphasize the differences between the control and hotspot soil profiles at depth, and the consequence of local surface disturbance on calculated ^{15}N enrichment at depth.

7) It would be interesting to see a plot % beaver derived N (as in Fig 4) vs. %N (or C:N) – this would provide additional evidence that the lower C:N ratios at the hotspots have developed due to beaver N inputs.

Response: Yes, we agree that this would be an interesting plot to generate, but we do not feel this plot is needed to provide additional evidence, and we do not have the data at present to accomplish this for soils at depth. Figure 3 shows that beaver-derived N (plotted as $\delta^{15}N$) influences soils up to 60 cm along the surface transects. The C:N values, while different within the hotspot (sample at 0 cm) compared to soil outside of the hotspot (soil at 140 cm), are not significantly different from control C:N values. There is an overall trend of lower C:N ratios within the hotspot, but because C:N does not significantly differ from control soils, we do not feel that graphing % beaver-derived N vs. C:N would add to our study.

8) Would it be possible to make an estimate of the total amount of beaver-derived N retained in the soils (under a carcass) and relate that to the total amount of initial beaver N? i.e., what fraction of beaver-N is retained in the soil after 1 year?

Response: Yes, this is a great suggestion. We have added this approximation to the discussion, based on the measured %N of soils relative to controls during the peak of decomposition and what was measured after one year. The text reads (Lines 279-284):

> "The total %N measured in soils can be used to approximate the contribution of beaver N to soil. During active decomposition, hotspot soils contained 36 % more N compared to control soils (0.362 % N vs. 0.267 %). After one year, hotspot soils still contained 10 % more N than control soils (0.285 % N vs. 0.260 %), reflecting a loss of ~28 % of the beaver-derived N in one year."

Technical comments:

9) L47-51: this section could be more specific (e.g. use "increase/decrease" instead of "change")

Response: The text was modified as suggested. We kept reference to pH shifts in soils during decomposition to "changes" because in some soils/experiments, pH increases, while in others it decreases.

10) L55: "insects and animals" - aren't insects animals too?

Response: Yes, the reviewer is correct. We replaced "animals" with "vertebrates".

11) L74-75: rather additional N inputs than enhanced reactions, right?

Response: Decomposition hotspots exhibit changes in N due to <u>both</u> additional input of N (and C), which stimulates soil microbial communities and results in enhanced reaction rates.

12) L85: what's the size of the carcass (cm diameter?) - I'm wondering how much of the 60 cm diameter enrichment was located directly under the carcass

Response: Figure 2 provides an image of the carcass and the extent of fluid migration (the decomposition island). The soil sampled at 60 cm was not beneath the carcass (we sampled perpendicular to the carcass).

13) L210-214: I think the main result is not a less positive slope, but rather that the linear relationship between log(%N) and d15N is lost. This makes a lot of sense as the natural processes that typically for the 15N depth gradient are masked by the recent input of 15N-enriched nitrogen.

Response: We agree that re-phrasing our observation as a loss of the linear relationship is more appropriate and revised the text. The reviewer articulated this observation well, so we also included the explanation provided by the reviewer in the discussion.

14) L222:"distinct isotopic enrichment" - rather distinct N sources. Enrichment is a process, not just the a differences in distinct N pools (see Z. Sharp's comments on isotope terminology https://digitalrepository.unm.edu/unm_oer/1/ chapter 2)

Response: We agree this is an important point to clarify. The text was modified as suggested, removing "distinct isotopic enrichment" and replacing it with "distinct N pools".

15) L297-299, 304-307: I don't really see much support for these claims for changes in biogeochemistry or discrimination in the data that is not explained by the mixing of two distinct N sources, so I would recommend removing these speculative sections.

Response: As we discuss previously in response to comment #2, there is agreement that within decomposition "hotspots" there are elevated rates of biogeochemistry, particularly N cycling. We agree that the initial input of an N source initiates changes to soil chemistry, subsequent responses by soil (and carcass-derived) microorganisms results in enhanced rates of N cycling. Given that there is support for the concept in the literature (see references cited in the response to comment #2), we do not feel that we are being overly speculative in invoking this explanation.

16) L316-318: This is a mis-interpretation of the poor linear relationship. The most shallow soil horizons have d15N value of 8.4 per mil. If these horizons contain a mixture of soil and beaver N, the beaver N source signature has to be larger than 8.4 (consistent with the endmember value used in the 15N mixing model.)

Response: Yes, we agree that this was a mis-interpretation (and too far-reaching) to include. We deleted the text.

References Cited

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