Dear Dr. Pantoja, 5 Sept. 2019

We appreciate the detailed and helpful comments you provided in addition to the three reviewer comments on our manuscript, "Spatial changes in soils table isotopic composition in response to carrion decomposition (BG-2018-498). We made all changes outlined in our responses to the reviewer comments, and describe below the changes in response to your comments. Please feel free to contact me if there are any questions regarding this revised submission.

Thank you,

Sarah Keenan

AE Comments:

1. Abstract needs major revisions (Reviewer 3´s comment). Lines 1-9 are a long introduction and it does not say why this issue is relevant. Results presented here are too general to evaluate extent of this influence (for instance how big of a change in $\boldsymbol{d}_{15}N$ *is observed, etc.). Lines 19-21 are not very instructive: a) "…potential to result in long-term changes to soil biogeochemistry…", it is not potential, It is up to a year and of 60 cm from hot spot (already said it in lines 17-18), b) "… and to contribute to bulk soil stable isotopic composition." (already said it in lines 16-17). Instead of repeating facts, I would add significance of findings of your work for the discipline.*

Response: We modified the abstract in response to Reviewer #3's comments, and many of the recommendations described above have been fixed. We added more specific details to the abstract describing the results of this study, and removed several lines of background text that were unnecessary and did not frame the study properly. With respect to the comment above about the text "potential to result in long-term changes", we retained this wording because currently, this is our knowledge. We know that in some soils/climates/environments, there have been clear demonstrations of long-term changes. However, this has not been demonstrated in all environments/soils, and has not been temporally resolved or spatially resolved adequately.

2. Lines 40-41. Replace microfauna in "soil microfauna (i.e., bacteria, fungi, nematodes)" since fauna refers to animals and bacteria and fungi are not.

Response: We retained microfauna (for nematodes) and revised the text to read: "microfauna and microbiota".

3. Figure 3. Label of dark circle should be sample instead of "Hotspots"

Response: Label edited as suggested.

4. Paragraph of lines 236-239 repeats information from previous lines.

Response: This text was modified in response to a reviewer's comments, and there is no longer repetition of information in the two sentences.

5. Line 254. Is it really 65.9%?, not 66%?

Response: We used the value presented by the refence cited, but we agree that 66% is perhaps more appropriate and modified the text.

6. Line 285, "to the soil profile at depth ". Do you mean soil depth profile?

Response: We are referring specifically to the profile of the soil at depth. We removed "profile" and simply put "soils at depth".

7. Lines 285-287. "Decomposition hotspots, however, disrupt the expected pattern (Fig. 5), causing surface enrichment, and likely leave a lasting impact on soil stable isotopic composition." Explain what you mean with "likely leave a lasting impact on soil stable isotopic composition" since it is clear from Fig. 5 that below 10-cm depth there is no difference with respect to the control (except for one point at 30 cm depth with nitrogen stable isotopes.

Response: We are specifically referring to surface soils here, which are isotopically enriched and different compared to what is expected for surface soils. We are not suggesting that there are measurable changes at depth, rather that surface soils are disrupted from what is normally observed.

8. Conclusions. Please limit to conclusions of your work; Lines 323-324 and Lines 328-330 are not. Lines 331-336 are too speculative and not resulting from your data therefore do not belong to this section.

Response: We removed the text as suggested and include specific results/conclusions from this study.

Dear Dr. Pantoja, 20 August 2019

We received three positive and constructive reviews of our manuscript, "Spatial changes in soils table isotopic composition in response to carrion decomposition (BG-2018-498)". Below we address the comments and recommendations provided by the reviewers (original comments in italics, responses beneath). We feel the revised manuscript is improved from the original version as a result of these valuable suggestions.

The primary changes to the MS include:

- Removing the three end-member model at the suggestion of Reviewer #2 (Fig. 7), which did not add to the main conclusions of the original manuscript;
- Adding details to the discussion, particularly emphasizing the broader ecological consequences of persistent carcass-enriched soil; and
- Framing the two end-member mixing model more clearly.

Other changes to the manuscript in response to specific comments are detailed below. Please feel free to contact me if there are any questions regarding this re-submission.

Thank you,

Sarah Keenan

Anonymous Referee #1 (Received and published: 23 January 2019)

Overall the manuscript, "Spatial changes in soil stable isotopic composition in response to carrion decomposition," within minimal revision is a well written and a sound contribution towards understanding the spatial influence of pulsed organic nutrient inputs into terrestrial ecosystems from the deposition of carrion. Numerous studies have approached this subject but the geographic expanse and complexity of the resultant biogeochemical responses leaves ample room for investigation. This work helps to bridge the gap between previous studies through both the spatial layout of the observations and the utilization of isotope discrimination factors and $\delta^{15}N$ methodologies to tease apart the spatial extent of carrion influence within the soil profile.

Addressing the following concerns and comments will enhance the quality of this manuscript:

Specific comments:

1) Table 1: Insert note that defines N.M.

Response: Note inserted to table legend.

2) Perhaps note that control values do not have an error term due to being homogenized into a single sample.

Response: Note inserted to table legend.

3) The table caption or a note should include the statement about this data being from Keenan et al. 2018 except for the one year data. Also include a reprint permission statement in text (section 180) and with the table if required by either journal.

Response: A statement has been added to clarify that some of the data is derived from Keenan et al. (2018a), and clarifying that the bolded data are new.

4) The caption states that the letters indicate differences between samples but it is not clear as to between which samples the letters are referring to from the caption or from what was readily found in the text.

Response: We added text to clarify that by "between samples" we were referring to between samples within each measured dataset over time (i.e., comparing pH from each sampling timepoint). The text now reads: "Letters indicate hotspot soil samples within each measured dataset (i.e., pH) that were not significantly different based on One-way ANOVA ($p < 0.05$)."

5) Figure 3. Again the caption states that the letters indicate differences between samples but it is not clear as to between which samples the letters are referring to from the caption or from what was readily found in the text.

Response: Text was added to the caption to clarify that letters indicate the soil samples taken at discrete distances from the hotspot center that were not significantly different based on a one-way ANOVA with post-hoc testing.

6) Figure 5. Similar comment to Figure 3. Clearly there are differences signified with depth but it is not readily apparent what the difference is between A, AB, B, etc.

Response: As with Figure 3, the caption was revised to clarify what the letters were indicating.

Technical corrections:

7) Section 110: The equations as written may prove confusing to readers unfamiliar with isotope ratio calculations due to the use of the backslash as the division symbol both within the numerator and denominator as well as between. Perhaps something like 13C/12Csample ÷ 13C/12Cstandard would be better.

Response: The equation was modified as suggested.

8) Section 175: The following sentences seem to be restating a similar conclusion, "The pulse of nutrient-rich fluids resulted in significant changes to surrounding soil physio- chemistry (Table 1, Table S1). Soils exhibited long-term changes to physiochemistry following fluid degradation by soil microbial communities." Consider strengthening this paragraph by combining or differentiating these statements.

Response: The two sentences were combined for clarity and to eliminate redundancy.

9) Section 185: ". . . values around 80 cm of the hotspot" presumably should read "80cm from the hotspot".

Response: Text modified as suggested.

10) Section 200: Finesse this sentence a little bit to clarify that the 60 cm extent was beyond the carcass decomposition island. I believe that is what you are trying to state.

Response: The sentence was edited for clarity.

11) Section 235: The flow and the strength of the second sentence could be enhanced by revising the inclusion of ", here at least one year,". This is an important point that should specifically state that the results are for the given location, climate, soil, etc. and perhaps it would be better to give this its own subsequent sentence.

Response: The text was modified to emphasize that these results are specific for this site, a point we raise further in the discussion.

12) Typo - The third sentence, "The beaver carcasses used this study," should be "used in this study".

Response: Typo fixed.

13) Section 315: Typo - Sentence missing "a", "Based on the isotopic discrimination factor (D) for N in hotspot soils, a linear regression . . . . ."

Response: Typo fixed.

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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Macdonald, B. C. T., M. Farrell, S. Tuomi, P. S. Barton, S. A. Cunningham, and A. D. Manning: Carrion decomposition causes large and lasting effects on soil amino acid and peptide flux. Soil Biol. Biochem., 69, 132–140, 2014.

Metcalf, J. L., et al.: Microbial community assembly and metabolic function during mammalian corpse decomposition, Science, 351, 158–162, 2016.

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Michael Philben (Referee #3) (Received and published: 2 August 2019)

Keenan et al. use C and N stable isotope ratios to demonstrate that N derived from carrion can persist in the soil for >1 year, down to ~10 cm depth and up to 60 cm from the site of the carcass. This shows that these decomposition hotspots can have a surprisingly long-term impact on soil nutrient status and biogeochemistry, even after visible evidence of carrion has disappeared. Previous studies have examined this question, but the present study is unique in also examining the lateral and vertical extent of carrion-derived N after 1 year.

Overall I found the paper to be interesting, concise, and easy to read. The qualitative conclusion (that carrion N can persist in the soil for >1 year) is very well supported.

However, I think the explanation of some of the quantitative aspects should be improved before publication.

General comments:

1) Some issues with the mixing models:

1A) The 2-source mixing model assumes differences in d15N are caused only by mixing of sources and are not affected by diagenetic fractionation. As noted elsewhere in the manuscript, it's quite likely that the elevated N availability would result in additional nitrification and denitrification, which would increase the d15N independent of source mixing. This assumption should be stated and its potential influence on the quantitative results discussed.

> **Response**: The reviewer is completely correct, and perfectly summarized that two distinct but related processes are controlling $\delta^{15}N$ in these soils: input of an N-rich (and enriched) source and subsequent diagenetic fractionation (driven by microbes). We have included a sentence from the reviewer's comment above into the Discussion, and added a paragraph to more clearly state that our two member mixing model likely includes contributions from both the N-enriched carcass and subsequent diagenesis.

1B) Conversely, calculation of the isotopic discrimination factor (Figure 6) appears to ignore the impact of having a 15N-enriched source in the surface soils but not the deep soils. In other words, if the d15N depth profile is driven by distinct sources (as indicated by figures 4 and 7), then the slope in figure 6 does not represent the isotope discrimination factor.

> **Response**: We reworded any reference to "discrimination factor" for clarity and replaced it with "observed isotopic discrimination" to emphasize that we are not trying to make inferences about processes occurring, rather that the slope of these lines changes. As the reviewer mentions, this is driven by changes to N sources, rather than some underlying process.

1C) I was confused by the use of both a 2-end member and a 3-end member mixing model. I think I understand that the former is for comparison along the lateral transect while the latter is for comparing soil profiles. Some additional explanation would be useful.

> **Response**: The reviewer is correct—the two end-member mixing model is for the surface soils and the three end-member model was used for soil profiles. However, based on comments and critiques from both Reviewer #2 and #3, we elected to remove the three end-member mixing model from the manuscript.

2) The introduction states a goal of ultimately moving toward quantifying ecosystem impacts of carrion inputs (Line 71). However, there is little discussion of how the results could be scaled to contribute to the ecosystem level. Can you put in context how much N was added via carrion, how much remains in the soil after 1 year, and how much was lost from the soil? It seems like this should be a relatively simple calculation using the biomass and %N of the carrion and the N content of the soils. This would be very helpful for quantifying the importance of carrion in the ecosystem N cycle.

Response: This is an excellent suggestion. Text was added to the discussion to provide these details (Lines 279-284).

Specific comments:

3) Abstract: the abstract is heavily weighted toward background information rather than results and experimental design

Response: The abstract was edited to remove some of the background information and to include more results.

4) Lines 47-50: can you be more specific about the direction of changes observed (e.g. does pH consistently decline, etc.)?

Response: This was also brought up by Referee #2 (comment 9) and the text was modified to describe the direction of changes.

5) Lines 154-157: I'm confused about the inclusion of both shallow and deep control soils in the mixing model. Can you explain the justification for this approach in more detail?

Response: We removed the three end-member mixing model.

6) Lines 273-275: offer an explanation why carrion had not effect on d13C? (looks like the decomposition fluids had similar d13C as the surface soil)

Response: We provided a reference for Wheeler and Kavanaugh (2017), where the authors go into great detail explaining a lack of observed change in $\delta^{13}C$. We added a sentence to our MS to offer a brief explanation, guided by previous suggestions by Wheeler and Kavanaugh.

7) Table 1: indicate why the 1-yr samples are bolded. N.M.=not measured?

Response: Text was added to the figure legend explaining the significance of bolded data and N.M. abbreviation.

References Cited

Wheeler, T. A., and Kavanagh, K. L.: Soil biogeochemical responses to the deposition of anadromous fish carcasses in inland riparian forests of the Pacific Northwest, USA, Can. J. Forest Res., 47, 1506-1516, https://doi.org/10.1139/cjfr-2017-0194, 2017.

Spatial changes in soil stable isotopic composition in response to carrion decomposition

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Abstract

Decomposition provides a critical mechanism for returning nutrients to the surrounding environment. In terrestrial systems, animal carcass, or carrion, decomposition results in a cascade of biogeochemical changes. Soil microbial communities are stimulated, resulting in transformations of carbon (C) and nitrogen (N) sourced from the decaying carrion soft tissues, changes to soil pH, electrical conductivity, and oxygen availability as microbial communities release CO_2 and mineralize organic N. While many of the rapid changes to soil biogeochemistry observed during carrion decomposition return to background or starting conditions shortly after soft tissues are degraded, some biogeochemical parameters, particularly bulk soil stable $\delta^{15}N$ isotopic composition, have the potential to exhibit prolonged perturbations, extending for several years. The goal of this study was to evaluate the lateral and vertical changes to soil stable isotopic composition one year after carrion decomposition in a forest ecosystem. Lateral transects extending 140 cm from three decomposition "hotspots" were sampled at 20 cm intervals, and subsurface cores were collected beneath each hotspot to a depth of 50 cm. Bulk soil stable isotopic composition ($\delta^{15}N$ and $\delta^{13}C$) indicated that one year after complete soft tissue removal and decay, soils were significantly ^{15}N-enriched by 7.5 ± 1.0 ‰ compared to control soils up to 60 cm from the hotspot center, and enrichment extended to a depth of 10 cm. Hotspot soils also contained 10 % more N compared to control soils, indicating that decomposition perturbs N pools. Our results demonstrate that carrion decomposition has the potential to result in long-term changes to soil biogeochemistry, up to at least one year after soft tissue degradation, and to contribute to bulk soil stable isotopic composition.

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1 Introduction

Nutrient hotspots form from the introduction of carbon (C) and nitrogen (N)-rich compounds into an ecosystem, resulting in elevated reaction rates compared to surrounding regions (McClain et al., 2003). For terrestrial and aquatic systems, hotspots may be sourced from fallen trees (Lodge et al., 2016), annual deposition of deciduous leaves (Vidon et al., 2010), animal scat (Erskine et al., 1998; van der Waal et al., 2011), or animal carcasses (Parmenter and Lamarra, 1991; Carter et al., 2007; Wheeler et al., 2014; Wheeler and Kavanagh, 2017). Hotspots sourced from animal carcasses, also referred to as carrion hotspots, significantly alter surface and belowground soil physiochemistry and plant communities in terrestrial ecosystems (Carter et al., 2007; Keenan et al., 2018a). These alterations can have significant long-term impacts; for example, large animal carcasses had measurable effects on a prairie ecosystem for at least 5 years (Towne, 2000), and a decade or more in the Arctic (Danell et al., 2002). In addition to providing a critical source of C and N, carrion hotspots are important sources of ecosystem heterogeneity (Towne, 2000; Bump et al., 2009b) and promote biodiversity (Barton et al., 2013).

Carrion decomposition occurs in a series of physical (Payne, 1965) and biogeochemical (Keenan et al., 2018a) stages. The breakdown and release of animal tissues provides a labile source of nutrients for insect and vertebrate scavengers as well as soil microfauna and microbiota (i.e., nematodes, bacteria, fungi). Studies evaluating the consequences of carrion decay on soil biogeochemistry have monitored decomposition on a range of timescales, from days (Metcalf et al., 2013; Macdonald et al., 2014; Keenan et al., 2018a; Szelecz et al., 2018) to years (Towne, 2000; Bump et al., 2009a; Keenan et al., 2018b), and in different climatic and geographic settings, including temperate forests (Melis et al., 2007; Cobaugh et al., 2015; Keenan et al., 2018a) and Australian rangeland (Macdonald et al., 2014), as well as under controlled laboratory

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settings (Carter et al., 2008, 2010). Some of the key changes that occur in soils following the deposition and decomposition of carrion include: changes to pH (both increases and decreases), increased electrical conductivity, decreased oxygen availability, increased gas fluxes (CO_2, CH_4, N_2O, H_2S), elevated rates of microbially-driven C and N cycling, and increased dissolved compounds available to microbes (NH_4^+, NO_3^-, Ca^{2+}, SO_4^{2-}) (Melis et al., 2007; Aitkenhead-Peterson et al., 2012; Keenan et al., 2018a).

Many of the rapid, pulsed perturbations to soil C and N pools observed at carrion hotspots, such as elevated microbial respiration rates (measured as CO_2 release) and changes to soil pH, return to background biogeochemical conditions during the skeletal stage of decomposition, when soft tissues have been largely or completely degraded by insect and vertebrate scavengers (Cobaugh et al., 2015; Keenan et al., 2018a). However, certain biogeochemical measures, including soil stable $\delta^{15}N$ composition, have been observed to remain enriched in soils collected at carrion hotspots compared to background soils for a protracted period of time, up to several years (Bump et al., 2009a ; Wheeler and Kavanagh, 2017). Soil stable isotopic composition integrates all biogeochemical activity within the soil as well as inputs from plant or animal matter. In contrast with $\delta^{15}N$ enrichment, no changes in soil $\delta^{13}C$ composition have been observed in surface soils of decomposition hotspots (Wheeler and Kavanagh, 2017; Keenan et al., 2018a). A variety of studies have demonstrated the potential for natural abundances of ^{15}N to be used as a tracer of ecological processes, including N input from animals (urea and feces) in N-limited and isolated ecosystems (Erskine et al., 1998) and input of marine taxa (salmon carcasses) to terrestrial and riparian areas (Kline et al., 1990; Koyama et al., 2005).

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While ^{15}N enrichment due to carrion decomposition has been demonstrated in previous work, these studies were limited to surface soils (maximum sampling depth of 10 cm) from the center of the hotspots (Bump et al., 2009a; Wheeler and Kavanagh, 2017). This has left a gap in our understanding of the spatial extent of carcass enrichment, which is ultimately necessary for quantifying ecosystem impacts of these decomposition inputs. Given the potential for natural abundance ^{15}N to serve as a long-term tracer of decomposition processes, the goal of this study was to evaluate spatial changes in stable ^{15}N-enrichment at a carrion hotspot one year post-decay. In particular, the lateral and vertical extent of stable isotope changes as a result of enhanced biogeochemical reactions in a hotspot is largely unknown. Soils beneath and adjacent to former carrion hotspots (up to ~40 cm, the extent of visible fluid migration) were expected to remain ^{15}N-enriched one year after decay. Additionally, isotopic enrichment was expected to persist to at least 10 cm depth, the maximum depth examined in previous studies.

2 Materials and Methods

2.1 Study area and sample collection

The study site was a mixed deciduous forest in East Tennessee (36°0'1.0" N, 84°13'1.6" W, ~330 m elevation). Soils were part of the Fullerton-Pailo complex and characterized as Typic Paleudults (Soil Survey Staff, 2018). The A horizon extended to approximately 20 cm depth. Five ~23 kg nuisance North American beaver (*Castor canadensis*) carcasses were placed frozen within scavenger prevention enclosures (1.19 x 0.74 x 0.81 m) and allowed to decay naturally, starting 31 July 2016. As part of a separate study, approximately 75 g of surface soil (0-5 cm depth) was collected a total of five times during decay beneath each animal (Keenan et al., 2018a). For this study, soils were collected on 8 August 2017, one year after decomposition, and

after bones had been removed from the site. Soils were taken from surface transects as well as from cores obtained at depth below three carrion hotspots. Approximately 30 g of soil from the top 0-5 cm were collected using a 3 cm diameter auger within the hotspot—an elliptical area 40 to 80 cm in diameter of visibly discolored soil (Figs. 1, 2). Surface samples were additionally collected along a linear transect radiating perpendicular from the longest axis of the elliptical hotspot at 20 cm intervals up to 140 cm (Fig. 1). Within the hotspots, soils were cored to a depth of 50 cm using a 10 cm diameter auger; cores were partitioned into depth intervals of 0-5, 5-10, 10-15, 15-20, 20-30, 30-40, and 40-50 cm depth (Fig. 1).

All soil samples were homogenized to a uniform consistency in the field by hand (changing nitrile gloves between samples), removing any rocks, roots, leaves, or vegetation larger than 2 mm. Samples were transported to the lab and processed immediately. Aliquots were oven-dried in triplicate at 105°C for 48 h to determine gravimetric moisture (Table 1, Table S1). Once dried, subsamples were powdered in an agate mortar and pestle and stored in 1.5 mL tubes until subsequent isotopic analyses. Samples (~25 mg for surface soils; ~50 mg for soils below 20 cm depth) were transferred into 5 × 9 mm tin capsules (Costech). Isotopic analyses were conducted at Washington University in St. Louis. Samples, standards, and blanks were loaded into a Costech Zero Blank autosampler and combusted in a Flash 2000 elemental analyzer. Soil $\delta^{13}C$ and $\delta^{15}N$ values were measured on a Delta V Plus continuous-flow (Conflo IV, Thermo Fisher Scientific), isotope-ratio-mass spectrometer. Standards included millet and acetanilide. Millet was used to evaluate linearity. Sample carbon isotopic values were corrected for sample size and instrument drift using millet and acetanilide, and nitrogen values were corrected using millet, acetanilide, and urea. Analytic precision was <0.2 ‰ for both carbon and nitrogen. Results are presented in δ notation as parts per mil (‰) where $\delta^{13}C = [((^{13}C/^{12}C_{sample} \div$

$^{13}C/^{12}C_{standard}) - 1) \times 1{,}000]$ and $\delta^{15}N = [((^{15}N/^{14}N_{sample} \div {}^{15}N/^{14}N_{standard}) - 1) \times 1{,}000]$. Vienna Pee Dee Belemnite was used as the carbon standard and air was used for nitrogen.

Soils collected from the center (0-5 cm depth) of all five hotspots and a composite control sample (pooled soil collected from five locations ~3 m from each hotspot) were also analyzed for microbial respiration rates (as evolved CO_2), ammonium, nitrate, nitrification potential, pH, electrical conductivity, dissolved organic C and N, and protein content, building from a previous study at the site and following the methods described by Keenan et al. (2018a). In brief, headspace CO_2 was measured immediately after placing and sealing soil into 60 mL serum vials, as well as after 24 h (LI-820, Licor Inc.). Vacuum-filtered (1 µm; Ahlstrom, glass microfiber) soil extracts (10 g soil: 40 mL 0.5 M K_2SO_4) were collected after shaking for 4 hours at 150 rpm at room temperature, and were frozen at -20°C until subsequent colorimetric analysis of ammonium and nitrate (Rhine et al., 1998; Doane and Horwath, 2003). Aliquots were oxidized with a persulfate solution to measure dissolved organic carbon (DOC) as evolved CO_2 and dissolved organic nitrogen (DON) colorimetrically as nitrate (Doyle et al., 2004). Nitrification potential was determined colorimetrically using a modified chlorate block method optimized for microplates (Belser and Mays, 1980; Keeney and Nelson, 1982; Hart, 1994). Soil pH and electrical conductivity were measured from a soil slurry (3 g soil: 6 mL deionized water) using a handheld multi-parameter meter (Orion A329, Thermo Scientific). Protein content was determined using the Bradford Assay (Wright and Upadhyaya, 1996; Redmile-Gordon et al., 2013). Because the goal of this study was to focus specifically on stable isotopes as long-term tracers in carrion hotspots, only surface soils from the five remnant hotspot centers were processed for full physiochemistry.

2.2 Stable isotope analyses

The contribution of carcass-derived nitrogen to bulk soil stable isotopic composition in surface transects was determined using a linear two-member isotope mixing model (Wheeler and Kavanagh, 2017; Keenan et al., 2018a), using bulk control soil $\delta^{15}N$ composition (0.1 ‰) as one end-member and beaver decomposition fluid (10.2 ‰) as the other. Decomposition fluid is the by-product of microbial and autolytic processes acting on a carcass after animal death. Fluids consist of amino acids, dead and live microbial cells, urea, water, and lipids, and represent one of the primary mechanisms for return of host's tissues to the surrounding environment. Decomposition fluid isotopic composition was previously determined, using fluids collected from three decomposing beavers left on a shallow plastic tray to intercept fluids (Keenan et al., 2018a). The linear equation for the isotope mixing model (Wheeler and Kavanagh, 2017) was defined as:

$$CDN = [(TEM - SEM)/(FEM - SEM)] \times 100$$

Where CDN is the carcass-derived N (%), TEM is the average $\delta^{15}N$ of soil from the treatment condition (sampling interval along the surface transects), SEM is the end-member control soil stable isotopic composition (0.1 ‰), and FEM is the end-member isotopic composition of decomposition fluids (10.2 ‰). The contribution of control soil-derived $\delta^{15}N$ to measured treatment conditions was calculated by subtracting CDN (%) from 100 %.

To track changes in $\delta^{15}N$ between surface soil and soil collected at depth, $\Delta^{15}N$ values were calculated by subtracting the $\delta^{15}N$ value of soil at each depth from values obtained at the surface of the hotspot and control sampling locations. Negative $\Delta^{15}N$ values indicate that surface soils are ^{15}N-enriched compared to soils at depth (Martinelli et al., 1999).

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The contributions of multiple sources to bulk soil stable isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) were assessed using Stable Isotope Analysis in R (SIAR) using the simmr package (Parnell et al., 2010). Three sources were integrated into the modeling: (1) surface control soils, (2) decomposition fluids, and (3) control soils at depth (40 cm). Soil $\delta^{13}C$ and $\delta^{15}N$ composition at each depth were modeled to assess which source exerted a greater influence on bulk soil isotopic composition.¶

2.3 Statistical analyses

Data were analyzed using SigmaPlot to test for significant differences between treatments and controls. For both surface and depth transects, data from the three transects were treated as replicates for subsequent statistical analyses. Significance ($p < 0.05$) was determined based on one-way ANOVA analyses with Holm-Sidak post-hoc testing. Significant differences between control and hotspot soils were determined using paired t-tests at each sampling depth or transect interval using R (R Core Team, version 3.5.0).

3 Results

3.1 Surface soil biogeochemical changes during decomposition

During carrion decomposition, fluids sourced from the carcass were released into the surrounding environment (Fig. 2). The pulse of nutrient-rich fluids resulted in significant long-term changes to surrounding soil physiochemistry following fluid degradation by soil microbial communities (Table 1, Table S1). In particular, after one year of decay, soil pH was significantly lower than control, initial, and pre-decay soils ($p < 0.001$; $F = 59.317$). In addition, bulk soil $\delta^{15}N$ remained significantly enriched compared to control and starting soil isotopic composition ($p < 0.001$; $F = 27.948$). Other physicochemical parameters, including conductivity, microbial respiration, DOC, DON, ammonium, nitrate, and nitrification potential all returned to background conditions after one year. With the exception of the one year samples, data were previously published in Keenan et al. (2018a) and are included here for comparison.

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3.2 Lateral changes in stable isotopic composition

Soils were significantly ^{15}N-enriched within the visible carrion hotspot (mean soil composition 7.5 ± 1.0 ‰) and up to 60 cm from the hotspot center (2.2 ± 0.5 ‰) compared to composite control soils (0.1 ‰) (Fig. 3, Table S2) (paired t-test, p = 0.016). Soil $\delta^{15}N$ values gradually declined, reaching background abundance values around 80 cm from the hotspot center. In contrast, there were no significant differences between control and hotspot soil $\delta^{13}C$, and no differences as a function of distance from the hotspot center (one-way ANOVA, p = 0.464; F = 1.004). C/N ratios were lower within the hotspot and exhibited a gradual and significant increase with increasing distance from the hotspot center (Fig. 3). However, there were no significant differences between hotspot and control soil C/N.

The influence of carrion decomposition on soil stable $\delta^{15}N$ isotopic composition decreases with increasing distance from the hotspot (Figs. 3a, S1). Soil C/N composition follows a linear trend, increasing by 0.07 per cm from the hotspot center (Fig. S1). Based on linear two-member isotope mixing models, carcass-derived fluids exhibit a linear decrease in contribution to soil isotopic composition with increasing distance from the hotspot. Carcasses contribute to soil stable $\delta^{15}N$ isotopic composition up to 60 cm from the hotspot center (Fig. 4), an area that was beyond the decomposition island and was not visibly discolored (Fig. 2).

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3.3 Vertical changes in stable isotopic composition

Soil collected at depth beneath the three mortality hotspots was significantly ^{15}N-enriched compared to control soils up to 10 cm depth (Fig. 5). Surface hotspot soils were also enriched at 30 cm depth compared to the control. Control soils became more ^{15}N-enriched with increasing depth. There was no significant difference between control and hotspot soil $\delta^{13}C$ and C/N values,

and both exhibited the same trends with depth. Soils exhibited ^{13}C-enrichment with increasing depth and a decline in C/N ratios.

Control soils exhibited a strong positive linear relationship between the negative of the natural log of bulk soil %N and stable isotopic composition, reflecting decreasing N and C availability with increasing depth. Decomposition results in a shift in the observed hotspot soil N isotopic discrimination (*D*, or the slope of the linear regressions) (Natelhoffer and Fry, 1988), leading to a breakdown of a strong linear relationship compared to control soils (Fig. 6). *D* does not change for C isotopes in control or hotspot soils.

In general, soils exhibit a trend of increasing $\Delta^{15}N$ (the difference between soil $\delta^{15}N$ value at a specific depth and $\delta^{15}N$ at the surface) with depth, reflecting ^{15}N-enrichment in deep forest soils (Martinelli et al., 1999). Hotspot soils exhibited lower $\Delta^{15}N$ values compared to control soils, indicating little change in ^{15}N-enrichment with depth (Table 2). Control soils displayed increasing $\Delta^{15}N$ with depth, a pattern globally observed in forest soils (Martinelli et al., 1999) (Table 2). Combined with *D* (Fig. 6), hotspot and control vertical profiles have distinct N sources and exhibit distinct N pools with depth.

Soils collected from 0-5 cm exhibited a significant contribution from decomposition fluids (Fig. 5). As depth increases beyond 10 cm, there is no change to the proportional contribution of decomposition fluid to bulk stable isotopic composition. By 30-40 and 40-50 cm depth, hotspot soil $\delta^{15}N$ and $\delta^{13}C$ compositions are similar to control soils (Fig. 5), indicating limited, if any, input from decomposition fluids.

4 Discussion

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Soils associated with carrion decomposition hotspots retained biogeochemical markers of vertebrate decay at least one year after soft tissue degradation. Within the hotspots, soils remained ^{15}N-enriched compared to control locations. At this specific location and soil type, decomposing animals have the potential to exert long-term changes, here at least one year, on surface and subsurface soil stable isotopic composition. The beaver carcasses used in this study, which were between 20 kg and 25 kg in mass, resulted in measurable changes to soil biogeochemistry down to 10 cm depth and up to 60 cm away from the hotspot center, beyond the area that was visibly discolored from decomposition fluids. 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. Based on prior calculations, each beaver introduced approximately 0.47-0.79 kg N and 2.7-4.6 kg C to the soil (Keenan et al., 2018a). After one year, approximately 0.1-0.2 kg N derived from the carcass remains within the soil.

The linear two-member isotope mixing model indicates that the contribution of carcass-derived fluids to soil isotopic composition decreases with increasing distance from the hotspot (Fig. 4). However, the observed isotopic enrichment likely reflects a contribution of a ^{15}N-enriched input (carcass fluid) as well as subsequent diagenetic fractionation driven by soil microorganisms. It is likely that the elevated N availability provided by a decomposing carcass would result in additional nitrification and denitrification, which would increase the δ^{15}N independent of source mixing. Nitrification potential rates were elevated during earlier stages of decomposition, but were not different from control soils after 1 year (Table 1). The two-member

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mixing model assumes a simple mixing of soil and carcass-derived N, but diagenetic fractionation is also likely involved in the observed $\delta^{15}N$ patterns along the surface transects.

The contribution of carcass-derived N to soils at depth as well as laterally is influenced by a variety of physical and climatic variables. Here, decay occurred during the summer in East Tennessee, with an average high of 32.2°C for the month of August. Carcasses were exposed to measurable precipitation six out of 10 days after placement, preventing soft tissues from significant desiccation and supporting abundant blowfly larvae and other insect activity. Blowfly larvae migrating away from the carcasses on the surface and within the soil to pupate likely provided an important physical mechanism to distribute beaver-enriched N to surrounding soils. Blowfly larvae can move up to 10 m away from the carcass, and typically extend down into the soil up to 10 cm depth, depending on the soil substrate properties (Gomes et al., 2006). As blowfly larvae disperse, they have the potential to physically transport decomposition fluids acquired internally or externally during feeding, release excrement during migration, or die, leaving their tissues to degrade. An estimated 66 % of pupae that disperse to pupate die en route (Putman, 1977). Rainfall may have also contributed to the downward movement of decomposition fluids.

The temporal persistence of isotopic enrichment hotspots is currently unknown, but is likely to be ecosystem, carrion type, and carrion mass-specific. A larger carcass would be expected to result in greater lateral and vertical dispersal of carrion-derived fluids, as well as greater changes to ecosystem processes, because of the greater volume of decomposing soft tissue (Baruzzi et al., 2018). In addition, larger carcasses may host a larger and longer-lived insect community (Parmenter and MacMahon, 2009), including blowfly larvae, which may further nutrient dispersal and may impact a larger area. Bump et al. (2009b) observed elevated

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foliar $\delta^{15}N$ values in plants growing on sites impacted by deer carcass (~56 kg) decomposition at least 2.5 years after decay in a temperate hardwood forest, suggesting a long-lived hotspot signature. In some ecosystems, such as the Arctic tundra, isotopic enrichment is likely to persist for even longer based on perturbations to C and N surrounding muskox after 5 to 10 years of decay (Danell et al., 2002).

Increasing $\delta^{13}C$ and $\delta^{15}N$ values with depth in soils has previously been observed in a variety of soil types and climatic conditions (Natelhoffer and Fry, 1988; Martinelli et al., 1999; Billings and Richter, 2006). Changes to $\delta^{13}C$ with depth are due to progressive cycling of C through microbial biomass (Liang et al., 2017), where selective preservation and biochemical fractionation together lead to ^{13}C-enriched organic C in soil (Natelhoffer and Fry, 1988; Billings and Richter, 2006). While we observed a similar increase in $\delta^{13}C$ with depth, we did not see a significant change in ^{13}C as a result of carcass enrichment. Wheeler and Kavanagh (2017) similarly did not observe a change in soil $\delta^{13}C$ following carrion decomposition, which may be due to the degradation of carcass-derived C (and uptake by microbes and blowfly larvae) combined with elevated background C in soil compared to N.

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Increasing $\delta^{15}N$ values with depth reflects two broad biochemical processes leading to fractionation, both likely driven by microbial activities. First, the preferential excretion of ^{15}N-depleted compounds during catabolism and anabolism leaves the residual microbial cells and soil ^{15}N-enriched. Second, kinetic fractionation associated with gaseous N loss is also known to result in enrichment, depending on the microbial communities present and N mineralization rates (Evans, 2001; Robinson, 2001; Liang et al., 2017). Over time, as soil profiles develop, accretion of ^{15}N-enriched microbial cells, particularly fungi, leads to isotopic enrichment at depth (Billings and Richter, 2006). In contrast, plant and leaf litter are the dominant contributors to N pools in

surface soils in most temperate forest ecosystems (Vidon et al., 2010), resulting in surface soils that are isotopically-depleted compared to the soils at depth. Decomposition hotspots, however, disrupt the expected pattern (Fig. 5), causing surface enrichment, and likely leave a lasting impact on soil stable isotopic composition.

For systems at or near steady state conditions, the difference in isotopic enrichment between soils at depth and the surface ($\Delta^{15}N$) provides a way to compare soils from different geographic and climatic locations (Martinelli et al., 1999), and was used here to compare hotspot soils and those collected at control locations. $\Delta^{15}N$ values observed in the control depth profile are within the expected range observed in temperate forests worldwide (2.7 to 9.1 ‰) (Table 2). However, as a consequence of carrion inputs and decay, $\Delta^{15}N$ values are more similar to those observed in tropical forest ecosystems (1.1 to 4.3 ‰). In tropical systems, lower $\Delta^{15}N$ values are thought to reflect more open N cycling with elevated N losses (nitrification, nitrate leaching, and ammonia volatilization) under conditions of elevated total N inputs (Martinelli et al., 1999). Whether our observed changes in $\Delta^{15}N$ are due to elevated N cycling rates, disequilibrium effects across the soil profile due to changing N inputs from a system dominated by atmospheric dry and wet deposition of nitrate and ammonium to one with carrion-sourced N, or both, is not known.

The observed stable isotopic discrimination (*D* or the slope of the linear regressions) did not differ for control and hotspot soil $\delta^{13}C$ (Fig. 6), suggesting that C cycling and pools in soils one year after carrion decay are not altered. In contrast, *D* values for $\delta^{15}N$ were different between control and hotspot soils, which reflects a loss of the linear relationship and indicates distinct N sources for the two soil profiles. This also emphasizes that decaying carrion provide an important and potentially distinct N pool for soil ecosystems that have the potential to mask natural (background) processes that control soil profile ^{15}N gradients with depth. In addition, differences

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in *D* values between the two soils suggest that there may be less discrimination occurring within hotspot soils compared to control soils, likely due to the rapid input of an isotopically-enriched N pool (Evans, 2001).

Hotspot soils received the input of beaver-derived fluids (10.2 ± 0.4 ‰) (Keenan et al., 2018a) as well as soft and hard tissues (1.0 to 4.0 ‰ for beaver bone collagen from Minnesota; Fox-Dobbs et al., 2007). Stable isotopic composition of surface soils strongly suggests that decomposition fluids are a significant contributing source to bulk soil stable isotopic composition up to 60 cm from the hotspot center (Fig. 4), and $\delta^{15}N$ enriched values in the soil profile at depth also suggest some contributions up to 10 cm depth (Fig. 5). Beyond 10 cm depth, control soils and hotspot soils are indistinguishable, suggesting that decomposition fluids do not significantly influence soil stable isotopic composition. Rather, natural $\delta^{15}N$ enrichment due to soil accretion processes can explain the observed soil stable isotopic composition.

5 Conclusions

The decay of ~23 kg North American beavers resulted in rapid (within days) and long-lived (up to one year) ^{15}N-enrichment in forest soils up to 10 cm depth and ~60 cm distal. Observed ^{15}N-enrichment at depth and laterally is likely due to a combination of physical movement of fluids during decomposition and the transport of fluids by insects, particularly blowfly larvae. In this system, rainfall during decomposition may have also acted as a physical transport mechanism. While likely to be significantly influenced by carcass size, climate, and soil type, decomposition has the potential to exert long-lived influences on soil stable isotopic composition.

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Deleted: Based on the isotopic discrimination factor (*D*) for N in hotspot soils, linear regression line for hotspots soil results in a predicted starting N source of 4.8 ‰, in line with the expected value for fresh beaver tissues (Fig. 6). This suggests that the stable isotopic composition of soil $\delta^{15}N$, even one year after decay, may be a useful tool to infer starting animal tissue isotopic composition.

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Data availability

All data generated in this study are available in the Supplement.

Author contributions. SWK and JMD designed the experiments. All authors assisted with data interpretation. JMD and SMS provided financial, lab, and analytical resources. SWK and JMD prepared the manuscript with contributions from SMS.

Competing interests. The authors declare that they have no conflict of interest.

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Figure and table legends

Figure. 1: Schematic cross-section view of the locations of soil samples (stars) collected from each of three carrion decomposition sites. Dashed line represents the hotspot—the area of visibly discolored soil. Soils collected at depth extended to the B horizon. The visibly discolored area of soil due to carrion hotspot formation extended approximately 35-40 cm from the hotspot center along the surface and to a few centimeters depth.

Figure 2: View of a beaver after placement (a) and during advanced decay (b) to demonstrate the lateral migration of carcass-derived fluids during decay. Both photos are from the same animal, and (b) were taken during advanced decay (8 August 2016). Visible extent of fluid migration is outlined in the white dashed line.

Figure 3: Lateral changes in soil stable (a) $\delta^{15}N$ and (b) $\delta^{13}C$ isotopic composition and (c) C/N ratios extending from carrion hotspot centers. Soil was visibly discolored 35-40 cm from the center (here, 0 cm distance). Letters indicate soil samples taken at discrete distances from hotspot center that were not significantly different based on an ANOVA and Holm-Sidak post-hoc test, and asterisks denote significant differences between control and hotspot soils (t-test) at each respective distance. The dashed line represents control surface soil (0-5 cm) composition.

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Figure 4: Results of linear two-member isotope mixing distinguishing the contributions of soil and carcass fluid to bulk soil stable isotopic composition.

Figure 5: Stable isotopic composition and C/N ratios for soils beneath carrion hotspots (closed circles) and at a control location (stars). Letters indicate hotspot soil samples as a function of depth that were not significantly different based on post hoc testing, and asterisks indicate significant differences between control and hotspot soils at each depth interval (both based on one-way ANOVA, $p < 0.05$).

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Figure 6: Bulk soil stable isotopic composition and corresponding negative natural log %N (a) and %C (b) for hotspot and control soils with depth. Linear regressions were fit to hotspot and control datasets.

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Figure 7: Stable isotope mixing models for hotspot soils collected at depth. (a) The three potential sources contributing to soil $\delta^{13}C$ and $\delta^{15}N$ isotopic composition were used as inputs, and exert different contributions at each depth. (b) The proportional contribution of decomposition fluids changes as a function of depth.¶

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Table 1: Selected soil biogeochemical data during one year of decomposition. Letters indicate hotspot soil samples within each measured dataset (i.e., pH) that were not significantly different based on One-way ANOVA ($p < 0.05$) and Holm-Sidak post-hoc testing. Asterisks indicate significant differences between control and treatment soils. N.M. indicates parameters were not measured. Control samples were homogenized into a single representative sample and do not have standard deviations. Data, except for bolded 1 yr. post decay, were previously published in Keenan et al. (2018a).

	Sampling Date	Soil Gravimetric Moisture	pH	Conductivity (µS cm^{-1})	Dissolved Oxygen (%)	Total carbon (%)	Total nitrogen (%)	C/N	$\delta^{15}N$	$\delta^{13}C$
Initial	29 July	0.299 ± 0.012^{AB}	6.79 ± 0.1^{A}	47.83 ± 5.9^{AC}	98.5 ± 0.29^{A}	5.11 ± 0.101	0.295 ± 0.009	17.33 ± 0.25^{AC}	1.48 ± 0.23^{A}	-27.86 ± 0.08
Early	1 August	0.216 ± 0.034^{A}	6.86 ± 0.3^{A}	73.53 ± 32.2^{A}	N.M.	3.927 ± 1.08	0.245 ± 0.058	15.93 ± 1.7^{AC}	2.65 ± 1.66^{A}	-27.73 ± 0.42
Early control	1 August	0.219	6.82	36.78	N.M.	4.196	0.247	16.99	1.76	-27.56
Active	3 August	0.234 ± 0.056^{A}	8.64 ± 0.3^{B}	2150.48 ± 1282^{BC}	9.16 ± 7.89^{BC}	4.251 ± 0.798	0.362 ± 0.096	12.01 ± 1.34^{BC}	6.23 ± 1.50^{B}	-27.77 ± 0.30
Active control	3 August	0.160	6.68*	31.65*	98.6 ± 1.25*	4.159	0.267	15.58*	1.48*	-27.68
Advanced	9 August	0.286 ± 0.081^{AB}	8.78 ± 0.1^{B}	1233 ± 494^{C}	19.4 ± 31.2^{B}	4.000 ± 1.29	0.303 ± 0.080	13.07 ± 0.75^{BC}	8.72 ± 2.09^{B}	-27.64 ± 0.20
Advanced control	9 August	0.223	6.84*	43.42*	98.0 ± 0.57*	5.008	0.281	17.82*	1.26*	-27.77
Early skeletal	6 September	0.242 ± 0.070^{A}	7.58 ± 0.4^{C}	973.8 ± 211^{AC}	97.4 ± 0.84^{AC}	3.610 ± 0.839	0.293 ± 0.057	12.28 ± 0.74^{BC}	9.26 ± 1.54^{B}	-27.74 ± 0.30
Early skeletal control	6 September	0.121	6.84*	35.08	98.3 ± 0.50	4.023	0.259	15.53*	1.78*	-27.63
Late skeletal	9 December	0.271 ± 0.021^{AB}	6.93 ± 0.3^{A}	225.2 ± 84.8^{AC}	100 ± 0^{A}	2.668 ± 0.352	0.214 ± 0.030	12.51 ± 0.67^{BC}	9.25 ± 1.33^{B}	-27.41 ± 0.25
Late skeletal control	9 December	0.246	6.73	29.13	100 ± 0	2.084	0.136	15.37*	1.79*	-27.30
1 yr. post decay	**10 August**	**0.404 ± 0.027^{B}**	**6.10 ± 0.3^{D}**	**29.47 ± 7.6^{A}**	**N.M.**	**4.253 ± 1.07**	**0.285 ± 0.036**	**15.24 ± 3.49^{C}**	**8.42 ± 1.52^{B}**	**-27.67 ± 0.25**

1 yr. post decay control	**10 August**	**0.449***	**6.29***	**23.07**	**N.M.**	**4.36**	**0.26**	**17.08**	**0.05***	**-27.73**

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Table 1 (continued):

	Protein (mg g^{-1})	Microbial respiration rate (µg CO_2-C release gdw^{-1} day^{-1})	DOC (µg C gdw^{-1})	Ammonium (mg NH_4-N gdw^{-1})	Nitrification potential rate (mg NO_2 gdw^{-1} day^{-1})	Nitrate (mg NO_3^--N gdw^{-1})	DON (mg N gdw^{-1})	Accumulated Degree Days (ADD)
Initial	0.251 ± 0.051	50.9 ± 8^{A}	2.44 ± 0.2^{AC}	0.039 ± 0.01^{A}	0.181 ± 0.05^{A}	0.000 ± 0.0^{A}	0.283 ± 0.02^{A}	26.1
Early	0.200 ± 0.037	51.4 ± 18^{A}	3.44 ± 1.0^{A}	0.101 ± 0.06^{A}	0.237 ± 0.16^{A}	0.000 ± 0.0^{A}	0.718 ± 0.35^{A}	106.7
Early control	0.159 ± 0.004	35.2	2.35	0.011	0.167	0.000	0.242	106.7
Active	0.260 ± 0.025	300 ± 90^{B}	66.54 ± 40.4^{B}	2.49 ± 1.24^{B}	0.366 ± 0.09^{A}	0.001 ± 0.0^{A}	1.363 ± 1.4^{A}	160.3
Active control	0.225 ± 0.007	27.8*	2.52*	0.010*	0.163	0.000	0.205	160.3
Advanced	0.281 ± 0.072	162.8 ± 110^{A}	42.5 ± 30.0^{C}	2.29 ± 1.80^{BC}	0.517 ± 0.17^{A}	0.001 ± 0.0^{A}	1.906 ± 1.7^{A}	321.7
Advanced control	0.239 ± 0.024*	45.7*	3.44*	0.015*	0.181	0.003	0.304	321.7
Early skeletal	0.238 ± 0.049	76.9 ± 42^{A}	14.3 ± 3.4^{AC}	0.775 ± 0.14^{AC}	8.57 ± 4.4^{B}	0.309 ± 0.169^{B}	6.089 ± 1.24^{B}	1042.8
Early skeletal control	0.193 ± 0.021	20.8	2.89	0.006	0.130*	0.000*	0.185*	1042.8
Late skeletal	0.250 ± 0.014	57.2 ± 26^{A}	10.2 ± 8.4^{AC}	0.246 ± 0.06^{A}	0.017 ± 0.20^{A}	0.019 ± 0.001^{A}	1.929 ± 0.37^{A}	2591.7
Late skeletal control	0.195 ± 0.016	46.5	3.12	0.012	0.039	0.000	0.309	2591.7
1 yr. post decay	**0.239 ± 0.021**	**64.1 ± 8^{A}**	**2.81 ± 0.5^{A}**	**0.008 ± 0.0^{A}**	**0.006 ± 0.00^{A}**	**0.001 ± 0.0^{A}**	**0.095 ± 0.01^{A}**	**6377.5**
1 yr. post decay control	**0.195 ± 0.008**	**59.5**	**2.43 ± 0.0**	**0.007**	**0.006**	**0.000**	**0.093**	**6377.5**

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Table 2: Differences in soil $\delta^{15}N$ at depth and $\delta^{15}N$ in surface soils for hotspot and control depth profiles.

	$\Delta^{15}N$ (‰)	
Depth (cm)	**Hotspot**	**Control**
0	0	0
5	-2.1	2.65
10	-1.5	3.2
15	0.1	6.6
20	0.9	7.7
30	0.9	6.1
40	1.2	8.4

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