Note: Reviewer comments are in black while our responses are in red.

Reviewer: 1  
  
Comments to the Author  
In the manuscript Effects of salmon inputs on soil nitrogen: implications for marine derived nitrogen subsidies to riparian areas, Feddern and co-authors analyze the soil nitrogen dynamics following an extensive 20-year nutrient subsidy manipulation in the riparian zone of a Southwest Alaska stream. The authors found an enriched del-15 nitrogen signal in the riparian zone, approximately the distance from the stream where the majority of carcasses were re-located. Furthermore, the del-15 N signal of soil ammonium was enriched as well. Their analysis provides a strong test of the two-source mixing model often applied to studies related to marine-derived nutrients, suggesting that additional considerations are important to make, lest the effect of MDN be overestimated.  
  
I found the results of the authors’ study of high interest. Furthermore, the paper was well written and presented the data in clear fashion. However, in my opinion, the article does not warrant publication in Ecology given the scope of the investigation. While the long-term manipulation of Hansen Creek is impressive, the authors find several conclusions that run counter to expectations (e.g., the data set violates the assumptions of marine-derived nutrient mixing models). The authors’ contributions to our understanding of MDN will surely inform and guide future studies.

But do these findings represent new knowledge in our understanding of ecological phenomena?

Or would the major conclusions of the paper been different had multiple time points been captured – or if compared with del-15 nitrogen values from a neighboring, unmanipulated stream at the same time point?

Our sampling strategy was intentionally aimed at addressing a specific question: “Do salmon nutrients change the fertility of soils over the long-term?” As a result, we sought to exclude known short-term changes that immediately follow the application salmon (or any fertilizer) to soils (sensu Holtgrieve et al. 2009). It is well established from numerous artificial fertilization studies that nitrogen (N) additions leads to *persistently* higher soil organic and inorganic N pools and transformations (mineralization and nitrification) when N is an important resource for the ecosystem. Furthermore, previous research and the magnitude of the experimental manipulation justifies our single pre-salmon sampling. The manipulation we describe was, on average, an addition 334.5 kg/ ha N per year for a continuous 20 years. This is a much higher application rate than most nutrient addition experiments that have shown persistent effects. For example, Strader and Binkley (1989) detected significant differences in soil N pools 15 years after a single fertilization of 470 kg/ ha N. A meta-analysis of long-term (a year or more) responses to fertilization found N addition increases background rates of soil organic leaching (461%), soil NO3- concentrations (429%), nitrification (154%) and denitrification (84%) (Lu et al. 2010). More modest increases in NH4+ and mineralization were aslo found.

Therefore, by examining the long-term response of the system (i.e., a year after salmon return as opposed to immediately after salmon return) we are measuring a proxy of overall N use and retention in the system, which can be confounded on the short term as increased N transformations and concentrations are accompanied by increases in N leaching from the system, meaning, it is not ultimately stored or utilized in soils and vegetation.

Nonetheless, we acknowledge that the original manuscript lacked some of this critical background information. We therefore have added a paragraph each to the Introduction and Discussion (lines 142-152 & 445-456) to clarify our approach and to add important context for readers from different disciplinary backgrounds.

Unfortunately, these questions that continued to come to mind as I read the manuscript cannot be solved with revisions. I feel the article will be quickly accepted at an alternative journal, but I do not consider the scope and breadth to be appropriate for Ecology.  
  
Given the quality of the writing and statistical analysis, I have few comments to provide to assist the authors in improving their manuscript.  
  
Specific comments  
L39-40: different hyphens and/or spacing used to express these three ranges

This has been modified.  
L42: no period after “al.” in citation

APA style does include a . after 'al', therefore the citations have been kept at 'al.' not 'al'  
L51: delete hanging comma after “salmonids”

Deleted.  
L79: delete extra space between “of” and “all”

Deleted.  
L202, 208: Inconsistent capitalization of sub-scripts in the equations and associated text

Corrected for consistency.  
L259-60: Why present maximum fractionation rather than the mean fractionation?  
We presented maximum fractionation because any fractionation that exceeds the salmon end member signature indicates the mixing model results are biased by not including soil processing. While mean is useful, we believe it is more useful to present the potential magnitude of the bias, which is represented by the maximum observed value.

Reviewer: 2  
  
Comments to the Author  
General comments  
The authors present data on soil properties and functions with a special focus on nitrogen as a response to a salmon carcass enrichment-depletion experiment that has been conducted for 20-years. The paper points out issues with the current interpretation of marine-derived nitrogen contribution to vegetation around salmon streams, and presents support for their argument. The authors measured not only the generally done bulk soil isotope values, but also those of inorganic nitrogen forms, thus looking at a finer resolution than other studies, and they combine the isotope data with functional measurements of nitrogen transformation rates. While I believe this is an important contribution to the knowledge on salmon influences on recipient ecosystems, there are a few points I would like the authors to consider.

Firstly, the use of the factors of bank (enriched vs depleted) and distance from stream (experiment limited to near-stream riparian area) as a basis to identify salmon influences may be problematic. While I understand and to some degree agree with this interpretation, it needs to be a) more clearly defined what “bank and distance” effect means, and b) discussed that other options also exist for a bank\*distance interaction. Looking at the models I only see models that include either distance or bank, or interaction terms. I am not clear on what exactly the difference is between the two interaction terms, why bank and distance are not included additive as well, or a combination of bank/distance effects in combination with interactions. As for other possibilities, nothing is mentioned on the soil types on the two banks, and which soil horizons are included in the sample. Soils can change greatly with distance from the stream and with aspect (warmer vs colder aspects can create different soils) which is indicated by the bank and distance effects on soil moisture (GW) for example, and different soils can have different isotopic signatures, as can different soil horizons.

We have modified our manuscript to 1) more clearly describe bank and distance effect 2) more clearly describe the interaction effects used and 3) have modified S1 and Table 1 to make sure these changes are conveyed to future readers.

We disagree with reviewer 2 that other options for bank and distance effects were not discussed. For each individual response variable that we tested we included in the discussion whether or not the responses we measured and the inclusion of an interaction term seemed reasonable to attribute to salmon or if it was more likely to be attributed to landscape effects. Specifically, we considered if the location of where the highest density of salmon was thrown (3m-6m) and whether it corresponded to elevated level of N concentration and transformation rates. We also discussed that temperature, soil moisture (line 418), and landscape position (line 110) are known to alter biogeochemical pathways and that landscape factors such as climate, aspect and water availability influence plant growth (lines 96-97). In fact, our results confirm these factors are important drivers of N transformations and concentrations, more so than salmon subsidies themselves (lines 417-420).

We have included additional justification for our statistical delineation of ‘salmon effect’ to include both bank and distance effects and have redefined our third hypothesis to reflect this. We do not believe additional discussion of landscape effects is necessary, however we do agree that reframing our distance and bank effect definition will help with clarity. These changes are described in more detail throughout the additional comments below.

Secondly, it is briefly mentioned in the discussion that the study was only done once, shortly before the salmon run in the main growing season of the terrestrial vegetation. I would like to authors to expand on this, especially in light of the 20 year experiment. How fast can nitrogen dynamics change in soils? How limited are soils for nitrogen, meaning how fast might a resource be used and thus not lead to accumulation? Can plants store nitrogen in the fall for later use? How much nitrogen would be ecologically relevant, meaning how much can we detect vs the uptake speed by trees?

Please see response to reviewer 1 comment 1 and additional details below:

If N from salmon is functionally important it should be retained in the ecosystem. This, in turn, should be identifiable as higher soil N concentrations and transformations even after a year after salmon fertilization. The reviewer fails to recognize that nutrients continually cycle between plants and soils and thus we should expect soil N pools to increase even if N is high demand by plants. As mentioned above, this is a consistent result from numerous fertilization studies across a wide variety of ecosystems.

Our findings that soil transformations isotopically fractionate N, biasing the frequently employed mixing models to quantify marine derived nitrogen, stand regardless of whether we are looking at long-term or short term effects. Both reviewers agree this is an important finding that should be considered going forward.

Finally, we note that the level of fertilization described here exceeds the application rate for the majority of nutrient enrichment experiments and is therefore indeed ecologically relevant. Despite this high level of enrichment, Quinn et al (2018) found that trees on the salmon-depleted bank were growing faster than the salmon enhanced bank, indicating the benefits we can expect from these subsidies are minimal and hard to predict at best.

Thirdly, how do the findings here apply to other studies on salmon effects on terrestrial ecosystems? The authors provide a great example on a study on the same system and how the assumption of no fractionation in soils can affect our interpretation of the importance of salmon nutrients. However, how would this affect our interpretation of salmon effects on terrestrial ecosystems in other areas? Would we expect such a high fractionation in other soils? What about other subsidies? In the introduction guano is mentioned, how would we re-evaluate results there? Would we need to? I think framing the study in such a broader context would be important to highlight the importance of the findings to the field of ecology, not “just” in terms of the salmon effects.

We would expect high fractionation in any system where significant soil N transformation are occurring and it is well known that N additions leads to increased N transformations. In fact, it has been broadly observed that ecosystems become enriched in 15N relative to 14N with increased additions of N due to evelvated N cycling (Craine et al. 2009). Therefore our findings should hold in general across all other salmon systems. Fractionation will be higher in systems with higher MDN and these fractionations will create bias MDN estimates.   
  
Specific comments  
  
Abstract   
Line 6 – did they really document the presence or simply show elevated 15N signatures?

Many direct consumption studies have documented presence via elevated 15N/

14N signatures and correlate it to salmon abundance or presence/absence. The salmon 15N/14N endmember sits well outside the range of other potential prey items for consumers. Therefore, equating high 15N/14N ***in consumers*** with more salmon derived N is reasonable. Our result show that it is more complicated in primary producers, specifically soils and terrestrial plants, and that a direct linear relationship (as is usually assumed for consumers but also applied to producers) is likely incorrect.

Line 16 – As one of the conclusions is that isotopic values are to be used with caution, I would maybe not say here that the isotopes CONFIRM MDN, but rather suggest, right?

Correct, it confirmed elevated 15N/14N but not necessarily MDN as elevated MDN can be caused by other sources of fractionation (i.e., mineralization and nitrification), as many previous studies have done. Lines 6 and 16 have been modified to reflect this. However, to be clear, we do not dispute that MDN is present in salmon systems nor do our results contradict this. What we do dispute is mixing models used to quantify the contribution of MDN to riparian systems, which is supported by our results.

Line 17/18 – delta values cannot show an input, correct? Values can show an increase that exceeds the assumed end member.

Correct, delta values cannot show an input, but if the delta value exceeds end members it shows that the end members are not reflective of potential N sources. This means that mixing models to quantify the proportion N from salmon are biased to predict a higher proportion salmon N than is actually the case.

Line 18: If rates are not higher, where does the fractionation originate? During a different time period perhaps?

Fractionation reflects nitrogen availability and the alpha value of chemical transformations not specifically the rate of those transformations. If there is more available nitrogen then the reaction will preferentially favor the heavier isotope (in most reactions) in this case 15N, in circumstances with more limited N then all of the N essentially ‘gets used up’ and less fractionation occurs. So no, the rate of mineralization or nitrification is not the driver fractionation and instead fractionation would be expected to be related to additional nitrogen availability. Ultimately, fractionation is determined by mass transfer of N through the soil N cycling system and the strength of fractionation effect (alpha of the reaction).

Line 21 – as you mention the vegetation, it would be important to know the relation to run timing.

This is described in detail in the methods section lines 158-161. We do not think this is necessary to have repeated in the abstract.

Line 22/23: so, what does this mean? Does N just accumulate? What about the greater finding that we might have overestimated the importance of MDN? I would suggest a stronger finishing sentence here.  
  
We agree overestimated importance of MDN is the key, and most useful finding and this sentence has been modified to reflect that. As figure 1 depicts, N can be leached or released into the atmosphere as N2O and N2 rather than accumulate.

Introduction  - Consider starting the introduction more broadly on the importance of subsidies in terrestrial systems, how they are measured, and that there might be pitfalls. Also, while the information about the importance of salmon to aquatic organisms is interesting, it is not really the focus of this study, nor are the bottom-up effects. I would suggest to rather focus on the terrestrial aspect, but make it broader to apply to subsidies beyond salmon as well.  
  
Line 29/30 – just as a note that might create confusion, MDN is often used in the literature as marine-derived nutrients, rather that nitrogen specifically.

We considered this, and have found in the literature that it refers to both depending the specific paper. It is particularly common for it to refer to marine derived nitrogen in papers that are exclusively measuring nitrogen stable isotopes, for example Helfield and Naiman 2001 and 2002, Hocking and Reimchen 2002. For the sake of space and repetitiveness we have chosen to define and apply it as nitrogen.

Line 45 – dead organic material is not really prey, as it is already dead, right?

Correct, we have modified line 45 to clarify.

Line 63 – this is a very specific example so I expected a citation here.

This is cited in line 66, Schindler et al. 2005. Lines 62-66 contain two sentences both of which refer to the same results and citation (as referred to by ‘this finding’ in line 64 at the start of the second sentence). We chose to cite it once rather than at the end of both sentences.

Line 106 – a third point here is that the signature of salmon is not assumed to vary, nor the nitrogen content of carcasses, which we know is not the case.

Generally salmon N and 15N is very consistent as shown in a metaanalysis by Johnson and Schindler (2009) which measured a mean of 11.09 per mil for sockeye salmon in southwest AK with a standard deviation 0.48. Other studies have found similar results (within 1 standard deviation of this result) in AK and other regions (Satterfeld and Finney 2002, Kaeriyama et al. 2004). Given strong homeostasis in vertebrates, elemental compositions do not change much. While we do agree that this is an important consideration, this variation of less than half a per mil likely has a relatively small effect on overall MDN estimates compared to fractionation occurring in soils (which can be 10s of per mil).

Line 117 – not sure what the “which” here refers to. The nutrient removal?

We agree the use of which is vague and detracts from the clarity of the sentence. It has been modified for clarity.

Line 134 – the mentioning of the long-time scales here is misleading, as the study is a snapshot in time. Consider removing here, or discussion more about timeframe, lags and such in the introduction.

Long timescales was meant to refer to the time scale of the manipulation as described as a knowledge gap earlier in the paragraph. We recognize how this may be misleading given we only measured one timeframe, and have modified the sentence to state “long-term manipulation” to clarify this.

Methods – The methods are well described but the “salmon importance” needs to be expanded more (see general comments). Also, make sure to detail how the stats were run (program, packages, …).

Packages and program were added in lines 222-223.

Line 155 - … represent typical … (delete of)

Of was deleted.

Line 156 – what does high spawning intensity mean here? The entire 2 km receive lots of fish? But I am sure there is inter-annual variation and the study was conducted before the run.

The entire stream receives a lot of fish every year (even in low abundance years), but there is year—to-year variability and carcasses accumulate at different abundances along the stream. This is described in detail in Quinn et al. (2018).. Spawning intensity was changed to “annual carcass abundance” for clarity.

Line 161 – so “only” the surface was sampled? Which soil horizons? How does this related to overall soil depth, and depth of tree roots?

It is common to sample the first 10 cm when measuring inorganic nitrogen concentrations and transformations because this is where most nutrient cycling occurs in soils, and both concentration and transformations decline exponentially with depth (Chapin et al 2002). A depth of 10 cm includes parts of both the O and A horizons. If we sampled deeper, we would not expect to see significantly different patterns in soil N pools a transformations.

To give the reviewer a frame of reference with one example, Owen et al. 2010 measured net mineralization in the O horizon ranged from was average to 13 mg N kg−1 day−1 and at depths of 15cm (M horizon) was only an average 2.1 mg N kg−1 day−1 and at 25cm was 0.15.

We have included further justification of our methods including an additional citation (Sparks et al. Methods of Soil analysis) for clarification.

Line 174: does this mean the sum of Nh4+ and NO3-? If so I would actually stated “…in inorganic nitrogen as the sum of …”.

We modified this to read “the sum of the total change in…” This is a rate, so it is the sum of the changes of both concentrations.

Line 176 – what about the conversion of NH4+ to NO3-?

The conversion of [NH4+] to [NO3-] is net nitrification which was described in line 175-176.

Line 222: interaction mentioned here, but there are two in the models. Explain why two interactions and how they differ.

We agree this needs more detail. Both interaction terms allow the effect of bank to vary with distance, one allows this relationship to vary linearly and the other allows it to vary quadratically (ie have a peak at 3-6m where salmon were most abundant). When fitting higher order interaction terms in models, you must include all of the terms from the highest order down to the linear term in the interaction. Otherwise, you force part of the model (whatever term you omit) to be exactly zero which imposes inflexibility to your model that causes bias. This explanation was not included as it is best practices to model fitting and seemed unnecessary.

We agree with the reviewer 2 that it is necessary to acknowledge both interaction terms, otherwise the model description is incomplete. We have modified line 222 to include this suggestion, and clarify to the reader the differences between these two terms.

Line 223 – river’s edge

Corrected.

Line 224 – but the interaction also means that the difference among banks are not the same at different distances, in addition to that pattern of distance not being the same on both banks.

Correct, this is what we were hoping to convey in line 224. The interaction terms allows the effect distance to vary by bank (and vice versa!). We added that “the effect of bank to vary by distance” to covey this correction

Line 229 – carbon has not really been discussed until now, so having delta 13C here was a bit strange. Also, add “for” before delta 15N.

“for” was added before delta 15N.

We were not explicitly studying carbon isotopes because we did not expect salmon to have a significant effect. Nonetheless, we did include these data since they are co-genetared during the 15N analysis. While they do not directly inform the hypotheses tested, we felt they do provide insight to overall landscape variability. Specifically, the lack of difference in 13C between banks suggests similarity between them. The strong variation with distance also suggests that proximity to the stream is an important consideration when selecting control sites. We believe the data is useful to report and not necessary to mention earlier.

Line 242 – the interaction could also be underlying landscape variability, as soils may differ between the two banks and with distance from stream.

We agree, which is why we discussed this possibility at length in the discussion. We also agree with reviewer 2 that this sentence is misleading and we have modified it to correct this. The section now reads:

“Therefore, model support for both distance and bank parameters and a peak response between 3-6m indicates an effect of salmon on the response variables, but support for only one of these parameters or no response at 3-6m demonstrates underlying landscape variability in the system… If models showed support for H3, the effect of salmon was confirmed by examining whether the response variable peaked at the salmon enhanced bank between 3-6m. If this did not occur, the response is likely due to landscape variability and not salmon.” In lines 244-247 and 257-259.

Line 245/246 – what does both a bank and distance effect mean? Both important as individual factors? An interaction? Individual with an interaction? Need to be very clear here.

See clarification from previous comment.  
  
Results – the results are pretty clearly presented. To help the reader I would use words rather than H1-4, such as bank differences, effects of distance, salmon effect, …  
  
Line 291 - … unlikely to HAVE impacted N transformation …

Edited.

Line 292 – this would actually be a good indicator that the soils are different between the banks and with distance, making the interpretation of the salmon effect questionable.

Our study is one of the only MDN studies that explicitly considers landscape factors and the assumption of biogeochemical similarity in soils of riparian systems. We believe biogeochemical similarity is immensely important for accurate MDN estimates from mixing models as discussed in lines 400-403 and 454-457.

We agree with this interpretation, which is why we interpreted almost all response variables to not have a measurable salmon effect with the exception bulk del15N and 15N of NH4+, which in addition to having support for a distance and bank effect, also peaked where the highest concentration of salmon were placed. Additionally, we agree landscape variability is important, which is why we discuss assumption 2, that most stable isotope mixing models assume control sites are biogeochemically similar despite not testing this similarity (and thus assumption 2 is likely violated).

We believe our modification of the hypotheses per the reviewers previous comments help clarify and rectify this.

Line 299 – I suggested to replace thus with “expected”.

Corrected.  
  
Discussion – The discussion is well-written but I am missing some topics that would frame the results in a larger context (see general comments). I think temporal patterns deserve some discussion, regarding expected lags, storage and reservoirs, accumulation of nutrients and so forth. Also, in the terrestrial, necrophile insects exist and can remove a large amount of the carcass material. How would that alter the numbers presented for input?

The calculations of g N from the manipulations, and potential shortcomings of these calculations are discussed at length in Quinn et al. 2018 where we cited the numbers from (including removal/predation by scavengers etc.) therefore we do feel it is necessary to duplicate their discussion here. Additionally, the goal of this work is to test how removal of salmon from a system may alter MDN and nutrient dynamics, insects and scavengers would have the same effect on natural salmon carcasses, so we don’t believe this discussion is necessarily relevant to the points presented or our results.

Line 316-318 – what if nutrients get used right away during the run? Salmon carcasses are very localized, but even under carcasses the effect disappears quickly.

See response to reviewer 1 comment 1.

Line 329 – I suggest to add “only thirdly” before nutrients.

Changed.  
Line 331/333 – don’t forget to add “δ”

Added.  
Line 338 – what does this 30‰ mean? That the created fractionation leads to a 30‰ difference? Or the values vary by 0.03%?

We are referring to fractionation as ‰ is the unit of measurement for fractionation, meaning it imparts a 30‰ isotope fractionation. We have modified this section to read “a fractionation up to 30‰” to clarify this.

Line 340 – why use however if the statement supports the idea presented above?

We agree and have removed ‘however’.

Line 344 – I suggest to add something along the lines of “leading to an overestimation of MDN importance”. It rounds of the idea and links to the next paragraph.

We agree and have modified the sentence per the reviewers suggestion.

Line 362-365 – what is the C pathway you are thinking of here?

Water stress is recorded in the carbon/isotope ratio of woody plants through the discrimination of 13C/12C through diffusion of atmospheric CO2 through the stomata at the site of carbon fixation by RuBisCO. This would effect soil del13C through litterfall and has been measured specifically in white spruce forests, discussed by Gabriel and Phillip 2016 and cited in this discussion.

Line 364 – replace from with to

Replaced

Line 405 – I think the ecological significance here should be expanded upon. How much N does a tree need? Are the differences we can measure actually relevant, and can we analytically even measure the difference in nutrient concentrations that could affect tree growth?

Yes, measuring nutrient concentrations using methods similar to ours are a common practice due to their ability to measure N on ecologically relevant scales. The amount of N trees require is highly variable and dependent on factors such as the age of the stand. Regardless, one example is a White Spruce in a floodplain stand requires approximately 1.47 g m-2 y-1 (Chapin et al. 2006) which is relevant to both the estimated numbers of grams of N from the salmon enhancement 33.45 g m2 y-1 and from our measurements. We agree this reference point may be useful and have modified lines 329 to 331 to include these estimates, however we do not believe it needs to be expanded on further.   
  
  
Table  
Table 1 – explain all the abreviations in the figure legend, especially I(ln(distance)2). Also, why does GW have distance as long10?

We have altered the table to include the covariates in the supported models. This, in addition to the added description of the interaction effects per reviewer 2s previous comment we believe the table is now much clearer. Log10 was a typo (should have been ln) and has been changed.   
  
Figures  
Figure 1 – the figure legend mentions isotopic fractionation, so why not include estimated fractionations for the different pathways? It would make the figure that much stronger to see how a pathway could mimic the salmon signature.

We agree this would be useful, however the specific fractionation imparted by a reaction is dependent on multiple factors:

1) the zero-point energy of the compounds at play 2) the relative availability of those compounds and 3) the alpha value of the specific chemical reaction (ie the arrows in the figure).

The specific fractionation we select would therefore be dependent on the amount of Nitrogen in the system and the microbial community processing that N which can impart drastically different fractionations. We hesitate to report a single fractionation value as it oversimplifies the process of fractionation.

Figure 2 – what are the predicted values? Those based on the models of Table 1? If so, which model was used?

The predicted values are based on the model with the most support from table 1 (bolded). We have modified the caption to clarify this.   
  
Supplemental Material  
Line 3 – value is misspelled

Corrected.  
Line 15 – add a comma after concentration.

Added.  
S2 – make sure to name all the variables in the heading.

Added.