

This file includes our original author response (prior to revisions) in full. Changes made to the revised manuscript (where needed) are added to the former response and highlighted in blue. These can be found by searching for “revision”.

Best,

Dave Bowling

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Thanks to the referees for sharing their expertise and their time. These thoughtful comments will help improve the manuscript should we have the opportunity to revise. As the process was explained to me, at this point we can only respond to comments but not submit a revised manuscript.

Dave Bowling, on behalf of all authors

Referee #1:

It is welcome relief to review a well-written manuscript on a timely and important topic. It is clear, logical and concise. Figures are well done. No major/minor comments or suggestions.

Response: Thanks for the very strong recommendation.

Referee #2:

This manuscript reports on a detailed experiment examining the stable isotopic composition of CO₂ in soil respiration and in soil air at various depths. The large dataset is used to evaluate the theoretical model based on steady-state diffusion theory and demonstrate that for the most part, the observations support the theory. The big surprise of this paper is that there were no diurnal or seasonal changes in the isotopic composition of soil respiration, in contrast to much previous work by the lead author and others indicating that autotrophic responses to environmental conditions can impose a physiological signal in the d¹³C of the respired CO₂. Indeed, the title of the paper points out this important observation, but most of the paper is focused on the comparison between observations and theoretical expectations. Unfortunately, the researchers do not have a very satisfying explanation for their unexpected results. Overall, the paper could be improved by considering why no variations were observed.

Response: It's true that at this point we cannot explain why we did not find evidence of environmental forcing on the isotope content of soil respiration, and we are open about that in the paper.

[Revision: We address this following the recommendation of referee 3, please see comments there.](#)

The introduction was well written, and provides a nice theoretical framework for the biophysical processes involved in d¹³C of soil respiration and production. This is a major strength of the paper.

Response: thanks.

The experiment was purportedly developed to test three main predictions (not really hypotheses), but the basis for these predictions is not completely clear. For instance, why were diel variations expected? In what way would they change?

Response: please see text on pg 6363 (line 27 and next few lines) and first paragraph of discussion.

Why would heterotrophic activity alter the $\delta^{13}\text{C}$ of soil efflux following rain, and not autotrophic activity? (indeed the authors have shown no effect of soil moisture on $\delta^{13}\text{C}$ in incubations as stated on p. 6377, L 23-25.)

Response: We agree that rain can affect both autotrophic and heterotrophic activity.

The importance of pressure pumping has also been well studied and it is not clear why it is set up as a hypothesis.

Response: Yes, pressure pumping has been well studied. Note that our data provide very strong evidence that pressure pumping in this soil is not significant (contrary to other studies and also to our own forest when covered with snow). The very fact that we found this result (which we did not anticipate) provides rationale for the hypothesis.

The discussion section includes a lot of background that might be better suited to the introduction in that it sets up the expectations/predictions (see below for more detailed comments).

Response: We agree, but feel that the discussion works much better with a merging of introductory material.

Although the research is rigorous, the authors could consider re-casting the objectives/hypotheses to better match the overall results of the manuscript, which is mainly focused on rigorously testing diffusion theory with a lot of data.

Response: Our perspective differs. Diffusion theory and CO_2 isotopes are not well-understood by the soil science ecological community, and a thorough introduction to these topics is really needed for people to understand the paper. However, our main focus was on environmental drivers and biological response to them.

The manuscript could have higher impact if it was shortened up to focus on the data, and to directly address (and explain) the unexpected results of no temporal variations in $\delta^{13}\text{C}$ of respiration or production. There is a nice discussion of autotrophic vs. heterotrophic respiration, and potential biological processes that should induce temporal changes in $\delta^{13}\text{C}$, but no real discussion of why these processes did NOT affect the

d13C in this study. Overall, the discussion should be shortened and focused to explain the results, and in so doing, should improve readability and impact.

Response: Referee #1 referred to this as a “well-written” manuscript that is “clear, logical, and concise”. Referee #3 wrote “The paper itself is well written; with rare exceptions (see below), the ideas are clearly expressed, well supported by explanations and evidence, and logically organized”, but agreed with some of your statements. Please see response to Referee #3.

Revision: [see response to referee #3](#)

Specific comments:

The methods section should explain that the soil gas wells collected a large volume of air (2.35 L of air, which would be equivalent to 4.7 L of soil volume if porosity was 50% and the soil was completely dry, or 9.4L if the air-filled porosity was 25% of total soil volume).

Response: If the Referee was able to write the above statement then the Methods section was successful in conveying the information!

This large volume equates to a radius of at least 10-15 cm, so samples collected at the O/A interface and 5-cm depth would be incorporating substantial air from above the soil. What is the influence of this large sampling volume on the model fits? Would the modelled profiles in Fig 7 be much different if the range of depths for each sample was increased (ie 10-cm depth sampled air from at least 0-20 cm)?

Response: please see detailed discussion of this effect on page 6380. Note that this is also highlighted in the last sentence of the abstract.

Restructuring the text in the Results section a little bit could improve the readability and streamline the manuscript to improve the overall impact of the paper. The paragraph starting on P. 6374, L 3 could be revised to start with a stronger topic sentence that conveys the main message of the paragraph, such as “The theoretical expectation for fully diffusive mixing between the CO₂ produced by respiration and the forest air was supported by most of the observations from the chambers, but not all of the gas wells (Fig. 6).” Then go on to explain the Keeling-type regressions and describe the main points in the paragraph. However, P. 6374, L 13-28 could be removed because these details are clearly presented in the table for those interested.

Response: this is a good suggestion and we will revise if we get the chance.

Revision: [Suggested sentence added at beginning of paragraph. We prefer to keep the text referred to in the last sentence of the referee’s comment because it helps to explain the results in Table 2.](#)

Figure 6: Please check the caption, the regression equation from the chamber inlet data seems to have a typo (+/- -26?).

Response: yes, that's a typo.

Revision: typo fixed

Similarly the following paragraph starting on L 24 could be restructured with a strong topic sentence of a main result, rather than just describing the figure caption. This will allow readers to more fully appreciate the importance of each figure.

P. 6375, L 6, please clarify that delta-J was calculated from gas well data, not “measured with gas wells”. Figure 7: The legends in part a and b are almost big enough to be able to read. In the caption, the horizontal dotted lines indicate the O/A interface and the top of the O horizon, because the O horizon (organic layer) is usually above the A horizon (mineral soil).

Response: good suggestions

Revision: 1) A topic sentence is problematic here. To keep this section brief, many different results are presented in this single paragraph in a just-the-facts way, and these are fleshed out more fully in the discussion. To take these several different topic sentences and separate each into its own paragraph doesn't make sense.

2) changed as suggested to “calculated from gas well data” 3) thanks for catching the O horizon typo, this is now fixed

Discussion:

P. 6376, L5-28, this first paragraph reads more like an introduction to why diel variation in $\delta^{13}\text{C}$ would be expected, and could be moved to the introduction. It concludes that no such variations were observed, but we are left wondering why not. Any ideas?

Response: We don't know why!

Revision: We address this following the recommendation of referee 3, please see comments there.

P. 6377, L. 4-29, similarly these two paragraphs provide a nice literature review (better suited to the intro) but again leaves us without even an educated guess about why no change following rain was observed.

Response: We don't know why!

Revision: see comments for referee 3

P. 6378, L4-16, this discussion of partitioning between autotrophic and heterotrophic components would be more appropriate if the paper presented direct observations of $\delta^{13}\text{C}$ in autotrophic and heterotrophic components. Based on the data, and considering the objectives of the paper did not address this topic, it is not clear why the recommendation on lines 14-16 is made. (likewise in the conclusions).

Response: We expected criticism over this. We feel that this is a significant point that needs to be made loudly. This work has been presented at both EGU and AGU meetings and many of our colleagues are in agreement. After decades working with CO_2 isotopes in ecosystem carbon cycling, we feel that the use of natural abundance C isotopes to

partition autotrophic and heterotrophic respiration is a pretty weak tool. We have another paper from a separate study (in review now in another journal) that addresses this in more detail.

P. 6379, Lines 3-29, considering that the gas wells sampled such large volumes of soil air, it seems likely that the pressure pumping effects could be challenging to observe.

P. 6380, L1-11, this first paragraph is a major strength of the manuscript; it could be the best dataset ever produced to illustrate the utility of diffusion theory to describe $\delta^{13}C$ in soil respiration.

Response: We very strongly disagree with the first statement, but agree with the second. Transport of gases in this soil is primarily by diffusion, and our study is a very rigorous demonstration of exactly that point. If pressure pumping were influencing transport we would find it because the isotope profiles are so sensitive to even minor advective influences – except at the shallow depths as we describe in detail.

P. 6380, L.12-29, in the opinion of this reviewer, too much emphasis is placed on the artifacts induced by pressure pumping from the shallow depths. It needs to be discussed but this section (as well as the results) could be shortened a bit. The recommendations to minimize gas sampling volume and to avoid disturbing the diffusion gradient are well supported, and could be quantified with some simple calculations indicating the depth range (radius) of the volume sampled.

Response: We disagree. In our roles as editor and reviewer for other journals we have constantly encountered soil CO_2 isotope studies where the authors simply don't understand diffusion and how easy it is to perturb the process. This is an extremely important message for the community. See next response.

P. 6381, L. 5-8, a recent study by Ogle and Pendall (*J. Geophys. Res. Biogeosci.*, 120, 221–236, doi:10.1002/2014JG002794) found the opposite (gas wells were more variable than chambers), possibly because the gas well volumes were much smaller. Please comment.

Response: The Ogle and Pendall study used a different chamber design that is certain to alter diffusive transport because it is non-steady state and the mole fractions in the chamber increase over time (see Nickerson and Risk 2009 GRL doi:10.1029/2008GL036945). The design will also cause pressure-based artifacts – changing the volume by subsequently closing multiple flasks in a closed-loop system will alter the pressure by an amount proportional to the volume ratios. In that design the perturbation will be a much larger pressure change than recommended to avoid artifacts by diffusion (see Fang and Moncrieff 1998 *Functional Ecology* 12: 319-325). Based on our experience this is not a suitable way to monitor the isotope composition of the soil surface flux, and hence it's no surprise that our results differ.

P. 6381, L. 9-18, this discussion addresses the main finding that was highlighted as the title of the overall manuscript, yet it is buried in a paragraph that starts out discussing

the difference between chambers and gas wells. If this section was moved higher in the discussion section it could help address the question why no temporal variations were observed.

Response: Agreed, this could be improved in a revision.

Revision: This paragraph really addresses the higher variability in the soil chamber observations relative to the gas wells, not the lack of response to environmental variation. We have modified the topic sentence to be more clear: “The $\delta^{13}\text{C}$ of the soil efflux measured in chambers was considerably more variable than the $\delta^{13}\text{C}$ of production observed using the gas wells (Fig. 8), but their means were quite similar”.

P. 6381, L18 to P.6382, L.7, this section could be condensed a bit. Start with a stronger topic sentence such as, “the isotopic composition of soil air in upper organic and mineral horizons is susceptible to advection, or pressure pumping, both due to natural weather dynamics and to methodological artifacts.” Then go on to (briefly) explain the details.

Response: Agreed, this could be improved in a revision.

Revision: We have added the suggested topic sentence. However, the description of horizontal pressure gradients in the soil and length scales associated with canopy and forest structure is quite important, and unusual in soil respiration studies, and we prefer not to cut this if the only justification is to condense.

Conclusions should be revised after considering the other recommendations provided above.

Response: It’s not clear how the conclusions should be revised from this comment.

Referee #3:

General Comments

This discussion paper, “Environmental forcing does not induce diel or synoptic variation in carbon isotope content of forest soil respiration”, by D. R. Bowling et al. nicely describes a very welcome observational study of carbon dioxide isotope production and transport within the soil. The study is well designed and thorough, providing quite a rigorous test of several commonly held ideas about the soil system (e.g. the three hypotheses that the authors set out to test). This is one of those clarifying papers that puts together not-so-novel pieces (e.g. chamber flux measurements, pore gas sampling, diffusion modeling) to give a view of the whole puzzle that is novel and valuable. I think this paper will be of much interest to the soil science community, as it was to me.

The paper itself is well written; with rare exceptions (see below), the ideas are clearly expressed, well supported by explanations and evidence, and logically organized. The figures are clear and effective as well. Personally, Figures 1, 6, and 7 were gratifying to see. How often are ecosystems so kind as to conform to simple mathematics?

Response: Thanks for the very strong recommendation.

I have had the luxury of reading the comments of Referees #1 and #2 before posting my own, and so I will add here that I am persuaded by two of Referee #2's general suggestions:

(1) Better addressing possible reasons for the surprising lack of variation in $\delta^{13}\text{C}$ with rain and time of day would indeed strengthen the paper. You seem to address the case of rain by arguing that there is no generalizable pattern in the literature, but then why did you seem to expect a $\delta^{13}\text{C}$ response to rain in the intro? For the lack of diel variation, you seem to blame heat conduction and diffusive transport (i.e. "strictly biological interpretations...are too simplistic"), but the logic is not clear: adding physical causes of diel variation on top of the biological ones seems unlikely to result in such a flat line as you observed. I don't expect you have the answers (and you don't need to for this paper), but some logical speculation or even just an explicit admission of mystery would help.

Response: We don't know why we did not find the expected variation. We are also disappointed that we can't explain it, but that's the truth. Given the very strong recommendations from Referee#1 and #3, and the generally supportive comments of Referee #2, we feel that this paper has considerable merit even without being able to explain everything. If we are given the opportunity to revise we will provide an "explicit admission of mystery" as suggested.

Revision: The editor recommended that we follow this strategy and we concur. We modified a sentence in the abstract: "Temporal variation in the $\delta^{13}\text{C}$ of the soil efflux was unrelated to measured environmental variables, and we failed to find an explanation for this unexpected result". The first paragraph of the discussion now ends with "Based on these results we reject our first hypothesis – there appears to be no diel variation in $\delta^{13}\text{C}$ of soil efflux at our study forest. We cannot explain why others have found such variation and we have not." The 3rd paragraph of the discussion now ends with "Hence there appears to be no generalizable pattern in the $\delta^{13}\text{C}$ of soil efflux following wetting, and we reject our second hypothesis. We do not know why others have found such high variation in $\delta^{13}\text{C}$ of soil efflux in response to wetting while we did not." Finally, in the conclusions, we write "We found no evidence for diel variation in the $\delta^{13}\text{C}$ of the soil efflux or of the CO_2 produced within the soil. We found no evidence that rain leads to changes in $\delta^{13}\text{C}$ of the soil efflux. We were unable to explain why others have found these patterns while we did not."

(2) The paper does have a bit of a split personality, with some aspects (e.g. title) being focused on the surprising lack of variation in the $\delta^{13}\text{C}$ of respiration (this result is about patterns in time) but most others being focused on the test of diffusion theory (this result is about patterns in space). Perhaps these two foci could be separated out better, e.g. with the results section first establishing the conformity to diffusion theory (excepting the sampling artifact) and then (perhaps in a second subsection) presenting the surprising time series results.

Response: A good suggestion.

Revision: After much thought we have decided to leave the results section as is, because we feel it is the most logical order of presentation, and allows us to focus from the beginning on environmental forcing. The discussion section is also focused from the beginning on environmental forcing, and we feel that these are among the most important aspects of the paper. We are certainly willing to revise further if the editor feels it is needed.

Specific Comments

Section 2.6: Unless you are restricting your determination of δ_F to nighttime data (and if that is the case then it should be noted in the manuscript), I believe you are assuming here that the isotopic signature of whole-forest respiration is identical to that of photosynthesis on the timescale of your study, i.e. that the isotopic disequilibrium is zero. The mixing line approach you cite, if including daytime data, should give the isotopic signature of the net ecosystem CO₂ source (i.e. NEE, not respiration) integrated over some time period preceding the measurement (that time period depends on the mixing time between the source and the background but is not precisely known). The signature of NEE will only be equal to the signature of respiration if the photosynthetic and respiratory signatures are identical. If you are making that assumption, then I think you should state it explicitly (and perhaps consider what error would result if the photosynthetic and respiratory signatures were actually different by, say, 1 permil, which I think is plausible).

Response: We use nighttime data only for this – it's described in full detail in the cited paper. We can certainly state this more clearly.

Revision: In section 2.6: "The $\delta^{13}\text{C}$ of whole-forest respiration (δ_F) was calculated for the entire study period from mixing lines (Ballantyne et al., 2011) between CO₂ and $\delta^{13}\text{C}$ of CO₂ in forest air at 9 heights, using nighttime data only".

page 6375, lines 19-20 (and page 6381, second paragraph): What is the measurement uncertainty for an individual measurement of δ_J , and what is the measurement uncertainty for an individual measurement of δ_R ? (These depend of course on the spectrometer uncertainty and on how δ_J and δ_R are calculated from the spectrometer data.) The differences in variability between δ_J and δ_R will only be meaningful if the variability is larger than the measurement uncertainty, but I don't believe you have shown that to be the case.

Response: The TDL isotope and CO₂ mole fraction uncertainties are 0.2 permil and 0.2 ppm. When propagated through eq 1 the uncertainty in δ_J is much less than 1 permil. Variability in the surface chamber fluxes (δ_R) is nearly 10 permil, and in the gas well δ_J calculations approaching 2 permil (Figure 8). Hence the uncertainty is minor relative to the actual variability.

very end of section 3: I don't understand the logic of "Due to the large number of samples, we do not interpret these small statistical differences as particularly meaningful". Shouldn't a large number of samples increase your statistical power and therefore make small differences more meaningful? The only reason I can think of to discount

a statistically significant difference (which you say you have found) is on account of some systematic error or uncertainty between the delta_R and delta_J methods. If you think such a systematic uncertainty exists, I think you should discuss and ideally try to estimate it.

Response: What we meant by that statement is that even though the statistical result says they are different, that doesn't provide useful information about ecological processes. One can imagine a study with far fewer samples that would come to the conclusion that these means were not significantly different.

Revision: End of section 3: "Due to the large number of samples, we do not interpret these small statistical differences as providing particularly meaningful information about ecological processes, with the exception of the O/A interface and 5 cm depths (as will be discussed later)."

Figure 3: Do you know why, after the first rain event causes it to step up, the respiration rate seems to step back down between the second and third rain events?

Response: The response to subsequent rain events after an initial wetting following a dry period is usually smaller (Borken and Matzner 2009 Global Change Biology doi: 10.1111/j.1365-2486.2008.01681.x)

page 6379, lines 16 ff: This is a very nice analysis of the contrast with the snow pack experiment.

page 6380, lines 12 ff: Here the relatively comprehensive nature of this study shines. It is great that you were able to discriminate between these two possibilities.

Response: thanks.

Technical Corrections

page 6363, line 8: "thus" implies that this sentence is a conclusion drawn from the previous sentences, but it is not (though the sentence is true). The previous sentence said soil respiration is the biggest flux from the terrestrial biosphere; this sentence says that the biosphere is important to predicting climate. I think in the previous sentence, you could say that soil respiration is the biggest flux of carbon to the atmosphere period (i.e. including anthropogenic sources), in which case this sentence would follow as a conclusion.

Response: agreed

Revision: Changed to "World soils are a major storage reservoir for carbon, and soil respiration represents the largest gross transfer of carbon to the atmosphere, much larger than anthropogenic sources (Le Quéré et al., 2013; Raich and Schlesinger, 1992). Understanding the complicated role of the biosphere in the global carbon cycle is thus essential for prediction of future climate (Friedlingstein et al., 2014; Heimann and Reichstein, 2008)."

page 6363, line 14: Similar to my previous comment, you write "as a result" but I don't

see how the fact that soil respiration is linked to plant photosynthesis implies that the residence time of the carbon in the soil efflux must be short.

Response: the wording could be improved but the point is correct

Revision: Changed to “In addition, there is strong evidence that soil respiration is linked to plant photosynthesis (Kuzyakov and Gavrichkova, 2010). Because of this, a large fraction of carbon in the soil efflux has resided in the biosphere for only hours to days to weeks (Högberg et al., 2001).”

page 6364, line 27: "biophysical" should be "physical" (the biology is in the production, not the transport of CO₂ within soils)

Response: agreed.

Revision: changed to “physical”

page 6365, line 3: need closing bracket after C_s

page 6365, line 16: "more" seems redundant

Response: agreed.

Revision: both changed as suggested

page 6369, lines 18-19: how are the 10cm diameter O/A interface wells inserted without digging?

Response: good point, they required some minor digging.

Revision: “Gas wells were used to monitor soil pore gas with as little disturbance as possible – no digging was required except for the shallowest that required minor digging.”

page 6370, lines 5-6: Do you mean that an individual measurement takes 10s, and 60 such measurements are made during the 10 min measurement period?

Response: no, the measurement takes 1 second, and 10 of those are averaged at the end of a 10-min period

page 6370, line 8: If gas flowed in each inlet for 10 min during measurement, then how could you measure 4 gas wells in 20 min?

Response: the inlet lines can flow without routing the gas to the analyzer with creative plumbing and a lot of initial headache

page 6372, line 12: should probably read "...of production, concentration of CO₂ in forest air, and δ¹³C of CO₂ in forest air..."

response: agreed

Revision: changed as suggested

page 6372, line 14: should probably read "described in the Results section" or "described in Results"

response: agreed

Revision: [changed as suggested](#)

page 6375, line 12: should read "however, that...", although I think this sentence is redundant with one in the next paragraph and so should be cut.

Response: agreed with wording, not the cut

Revision: [wording changed as suggested](#)

page 6376, line 5: all respiration is biological, no?

Response: Indeed, but here we are trying to highlight that there is a biological process that is sensitive to temperature. Many people refer to the soil surface efflux as "soil respiration" and that is disconnected from the biological production as discussed.

page 6382, line 11: should read "...of forest air – and compared..." (dash, not comma). Also, the list of methods lacks parallelism. How about "– soil surface chambers, soil pore gas wells, and forest air inlets –"?

Response: thanks for your attention to detail

Revision: [changed as suggested](#)

Figure 6 caption: I don't understand " $\delta^{13}\text{C} = 6997/\text{CO}_2 \pm 26 \text{‰}$ "

Response: a typo

Revision: [typo fixed](#)

Figure 7 caption: on the fourth last line, δ_R should not be inside the parentheses

Response: thanks

Revision: [corrected](#)

Figure 7 caption: in the second last sentence, I would write "Lines show the results of the diffusion model (see text) fitted to either all measurement depths..."

response: agreed

Revision: [changed as suggested](#)

Figure 7 caption: the last line should read "the top of the O horizon, respectively." (not A horizon)

Response: thanks

Revision: changed as suggested