Measuring and modelling the isotopic composition of soil respiration: insights from a grassland tracer experiment

U. Gamnitzer, A. B. Moyes, D. R. Bowling, and H. Schnyder

Author reply to comments of Reviewer #1

Reviewer #1: The manuscript "Measuring and modeling the isotopic composition of soil respiration: insights from a grassland tracer experiment" by Gamnitzer et al. is an innovative exploratory exercise to reveal physical mechanisms behind, what the authors describe as, transient dynamics in the flux of $\delta^{13}CO_2$ from soil. The information presented is highly relevant to the audience of Biogeosciences and especially for the audience of the forth coming special issue "Stable isotopes and biogeochemical cycles in terrestrial ecosystems". This article should be accepted for publication after the changes outlined below are implemented.

Author reply: We thank the anonymous referee for this remarkably comprehensive review (!) and the detailed suggestions. They helped to present our approach and findings more clearly. We considered the individual suggestions as follows.

Reviewer #1: Main comments:

I think the conclusions the authors draw about the modeling results are largely overstated. On page 100 line 24, the beginning of the discussion, the authors state they provide "direct" evidence for isotopic disequilibrium effects when, in fact, they provide the exact opposite. The results are indirect because the actual measurements of the system, especially of dissolved CO_2, were not taken. Related to this point, there are too many assumptions made in the modeling process for these results to be considered conclusive (I discuss below the assumptions made regarding static diffusion with depth, homogenous soil pH, and the "known" respiratory source). In order to provide direct evidence for the dynamics presented in this article, a sophisticated experiment would need to be designed with precise hypotheses. The results, however, are provocative, and the value of this research will primarily lie in methods development and formulation of new hypotheses. Therefore, in the revision the conclusions as currently stated should be de-emphasized.

Author reply: We agree that we did not directly measure the isotopic disequilibrium of CO_2 species in soil air and soil water. Still, we demonstrate that the proposed disequilibrium effects are sufficient to explain our observations. Thus, we reworded the Discussion accordingly, to avoid any ambiguity in this regard. The respective paragraph reads now "This work demonstrated that isotopic disequilibria in the soil CO_2 pool can explain the divergence between nocturnal ecosystem $\delta^{13}C_{\mathrm{efflux}}$ and ecosystem $\delta^{13}C_{\mathrm{Rs}}$ which was observed in a grassland tracer experiment. This $^{13}CO_2/^{12}CO_2$ flux disequilibrium appeared as a transient feature in closed chamber studies (in which the Keeling plot approach was used). A change of $\delta^{13}C_{\mathrm{atm}}$ at the beginning of the closed chamber Keeling plot measurements was shown to potentially induce the proposed disequilibrium."

Reviewer #1: I found the characterization of diffusion in the soil profile severely insufficient. There have been numerous articles published concerning the estimation of the diffusion of CO_2 in porous media (Moldrup et al., 2000; Hashimoto et al., 2002; Davidson et al., 2006; Resurrecion et al., 2008; Koehler et al., 2010; Vargas et al., 2011). First off, I think it is important to recognize that most of the reports on this subject are actually providing estimates of "effective diffusivity" – they are not direct measures. These models essentially rely on soil air porosity (as affected by soil moisture and temperature) and an estimate of tortuosity – the actual path of CO_2 is unknown. However, the authors provide no justification for the model they chose (Millington-Quirk) and they don't include in their discussion how D_{s} model choice might affect the D_{s} values they used in the respiration model and the subsequent impact on their allocation of variation in fractionation. The paper will be improved by considering multiple D_{s} models which should be included in the revision.

Author reply: In fact, numerous models describing the effective gas diffusivity in porous media have been published. Their predictions of the effective diffusivity of CO_2 in soil cover a wide range. Moldrup et al. (2000, 2004) and Hashimoto et al. (2002) showed that predictions derived with the Millington-Quirk model for the range of porosities observed at our experimental site, agreed well with measurements for soil conditions closest to those at our site.

However, a sensitivity analysis of our model results to the choice of a diffusivity model, as suggested by the reviewer, might be helpful to judge the validity of our conclusions. Therefore, we included further diffusivity models (Millington and Quirk, 1960; Moldrup et al., 1997, 1999, 2000, 2004) in the revision. In particular, sensitivity analysis is provided (Fig. 7).

Reviewer #1: I found the characterization of advection in the article to be misleading. The authors essentially deal with advection as an artifact of chamber methods (sensu Phillips et al., 2010). The simulation does not address wind pumping (Takle et al., 2003, 2004; Massman, 2006; Massman and Frank, 2006), advection as described by Lewicki et al. (2003) or as described by Camarda et al. (2007). In the revision, the discussion of advection should be limited to chamber measurement artifacts.

Author reply: The reviewer is right. Consequently, in the revision, we now refrain from discussing other sources of advection (wind pumping and geological contributions).

Reviewer #1: I think the authors need to be very careful in the assumption that the open chamber measurements of Gamnitzer et al. (2009) represent estimates that have not been influenced by "disequilibrium effects". It is also likely that many transient fractionation events are in effect, cancelling each other out (Nickerson and Risk, 2009). This introduces a level of uncertainty in the subsequent modeling estimates and general conclusions that is not really discussed in the paper. I recognize this is difficult to balance. One of the real strengths of this paper is the use of field data rather than a simulated dataset. However, for this paper, it is important to be transparent in the modeling process so that readers are able to judge the impact of the findings appropriately.

Author reply: Indeed, we cannot exclude that disequilibrium effects cancelled out each other in the open chamber measurements. But the concordance of these open chamber measurements with the reference measurements in the cuvettes of the laboratory-based system (Gamnitzer et al., 2009) supports the view that the open chamber measurements represent an unbiased estimate of $\delta^{13}C_{Rs}$. For our interpretation, it is irrelevant whether this unbiased estimate resulted from the absence of disequilibrium effects or from coincidental cancellation of opposing disequilibrium effect in the open chambers. However, it is unlikely that identical disequilibrium effects occurred in two different techniques (open and cuvette measurements).

Reviewer #1: The assumptions behind soil input parameters are another weakness in the modeling analysis, specifically, the assumption of static porosity and pH from the surface to a depth of 25 cm. I would expect the variability in porosity with depth to have a significant impact on the diffusivity of CO_2 at different depths, yet, the model treats the soil as an homogenous block. This criticism also applies to soil pH. The pH measurements in soil can be highly variable (Hinsinger et al., 2009), especially when considering the rhizosphere, ostensibly where we see much of the CO_2 exchange in and out of solution. Again, the manuscript does not address the impact of this assumption on the model results. Without direct measurements of the variability in pH the authors cannot support the conclusion regarding the importance of dissolution fractionation of $\delta^{13}CO_2$ that occurs during CO_2 and its subsequent impact on estimates of $\delta^{13}C_{Rs}$.

Author reply: In general, porosity and pH can strongly vary within soil especially at a very small scale (e.g., rhizosphere, intra-aggregates). We assume that this is not relevant at our scale and especially at our experimental site for two reasons. First, the soil was homogeneous due to regular tilling until few years before the experiment was started. Second, the site is calcareous, resulting in homogeneous carbonate equilibrium. Thus, the homogeneity assumption is acceptable for model application to our field site. For application to other sites, these model constraints need to be reconsidered. In the revised manuscript, we discuss this as follows:

"Several assumptions behind the model were chosen according to the specific conditions at this particular field site. The assumption of homogeneous distribution of pore size with depth is based on the past land use of the site as arable land, including periodic tillage. With conversion to grassland 8 years before the labelling experiment, differentiation of pore size distribution could have started. To account for this, the uncertainty range includes the observed variation in porosity between the soil surface (0–3 cm average) and a depth of 7–10 cm (Table 1). Similarly, depth variation in pH is neglected, in particular because the site shows calcareous characteristics with high buffering capacity. Variations of soil pH in the rhizosphere can be high, but are limited spatially (few millimeters around the growing parts of roots, see e.g. Revsbech et al., 1999) and temporally (within days, see e.g. Flessa and Fischer, 1992). Therefore, they were not considered in the present study."

Reviewer #1: General notes on manuscript construction:

In general, I found the manuscript fairly underdeveloped. I think the authors should take their time in crafting the manuscript so that readers may appreciate the work involved in this project. The introduction begins with a discussion of the isotopic signal of ecosystem respiration and the impact of the isotopic signature of soil respiration on these estimates. Then the following discussion relates only soil respiration issues, only to come back to ecosystem respiration at the conclusion of the introduction. This paper is primarily about soil respiration and I am not sure of the relevance of ecosystem respiration, especially since there is no reference to potential plant respiration effect throughout the text. While it is clear from Fig. 2. that there is an abiotic mechanism behind the depleted isotopic signal in respiration, it is difficult to discern what is actually being investigated (ecosystem or soil respiration).

Author reply: The reviewer is right. The disequilibrium effects which we discuss are related to soil respiration. Soil respiration accounts for a major part of ecosystem respiration, thus the disequilibrium effects also occur in ecosystem respiration. In grasslands, it is difficult to measure soil respiration separately from aboveground (shoot) respiration. Thus, we included shoot respiration in the model in order to make the model results comparable to the measurements from our field chamber. However, we assume that shoot respiration is not affected by the disequilibrium effects. We revised the manuscript as follows:

"The carbon isotopic composition (δ^{13}C) of soil respiration is often interpreted in terms of environmental and metabolic effects on soil carbon dynamics (e.g. Bowling et al., 2003; McDowell et al., 2004; Ekblad et al., 2005; Mortazavi et al., 2005). In general, δ^{13}C of the respiratory source ($\delta^{13}\mathrm{C}_{\mathrm{Rs}}$) is not measured directly, but is equated with δ^{13}C of CO_2 efflux ($\delta^{13}\mathrm{C}_{\mathrm{efflux}}$). However, soil CO_2 efflux can differ isotopically from concurrent respiratory CO_2 production due to transient conditions within the soil CO_2 pool. This divergence (termed 'disequilibrium effect' in the following) complicates the interpretation of $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$. Here we investigate mechanisms affecting this disequilibrium effect.

... Soil respiration accounts for a major fraction of grassland ecosystem respiration, thus disequilibrium effects in soils can generally affect the interpretation of the isotopic signal of grassland ecosystem respiration. Shoot respiration (the remaining fraction of ecosystem respiration) is not expected to produce comparable disequilibrium effects for carbon isotopes, since the relatively small CO_2 pool in leaf stomata is turned over much faster than the soil CO_2 pool."

Reviewer #1: Please spend some time on differentiating between the word use of disequilibrium effect and transient effect in the text. Often times you used transient as an adjective then later on as a noun (be careful with the use of the word transient as a noun because it also has another more common meaning). I think when you write "transient effects" you simply mean the system is not at steady state.

Author reply: In the manuscript, the term "disequilibrium effect" is defined as the difference between $\delta^{13}\mathrm{C}_{\mathrm{efflux}}$ and $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$ (see first paragraph of the Introduction section), and used in that sense. We agree that our use of "transient" as a noun was incorrect. Consequently, we replaced the noun "transient" by "transient condition".

Reviewer #1: I would suggest the use of $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$ (isotopic signature of the carbon source respired) and $\delta^{13}CO_2$ to distinguish between source and flux.

Author reply: We agree and replaced δ_{resp} by $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$ in the revised manuscript. Also, we replaced δ_{efflux} by $\delta^{13}\mathrm{C}_{\mathrm{efflux}}$ in the revised manuscript. In the same way, we replaced δ_{atm} by $\delta^{13}\mathrm{C}_{\mathrm{atm}}$.

Reviewer #1: Specific comments/recommendations

Abstract: Page 84, Line 19. Biogeosciences asks you to avoid using references in the abstract. This reference is unnecessary and should be removed.

Author reply: In the revised manuscript, we changed the corresponding text to "Earlier work has shown that the $\delta^{13}\mathrm{C}_{\mathrm{efflux}}$ measurements of the laboratory-based and steady-state systems were consistent, and likely reflected $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$."

Reviewer #1: Introduction: Page 85, Lines 14–15. Certainly there have been more investigations (especially in the field) on this subject: Susfalk et al., 2002; Millard et al., 2008; Kayler et al., 2008, Kayler et al., 2010b; Maseyk et al., 2009.

Author reply: These studies (and also others, e.g. Dudziak and Halas, 1996) complement the studies mentioned in our manuscript. By referencing to other studies, we want to stress that non-steady-state conditions in the soil under natural conditions have been previously observed. Accordingly, we included the respective studies and reworded the text as "Transient conditions in the soil diffusive system have been observed under natural conditions (e.g. Dudziak and Halas, 1996; Millard et al., 2008; Maseyk et al., 2009; Moyes et al., 2010)."

The studies by Kayler et al. consider slightly different aspects of transient conditions and are thus included elsewhere (see also reviewer comment regarding Page 87, Line 5) in the Introduction of the revised manuscript, reading "From a diffusion experiment involving artificial soil and CO_2 source, Kayler et al. (2008) concluded that non-steady-state effects must be considered in field investigations of $\delta^{13}\mathrm{C}_{\mathrm{Rs}}$ in soils. Furthermore, Kayler et al. (2010b) demonstrated in a field study the interrelation between perturbations of CO_2 in soil pores and aboveground measurement techniques for soil respiration."

Susfalk et al. (2002) investigated lateral changes of $\delta^{13}\mathrm{C}$ in soil air and soil efflux. They included month-to-month differences as results of a steady-state model, but no experimental data were shown. Hence, this work seems not directly relevant to transient conditions in terms of temporal changes as investigated in our present study, and we did not include it in the revised manuscript.

Reviewer #1: Page 85, Line 24. I thought Subke et al., 2009 was a quantitative explanation. Thus, this sentence and the previous contradict each other. Re-write lines 24–25 to clarify this.

Author reply: As far as we understand, Subke et al. (2009) measured $\delta^{13}\mathrm{C}$ of soil air CO_2 and soil CO_2 efflux, and quantitatively explained $\delta^{13}\mathrm{C}$ of CO_2 in soil air pores with a diffusion model. Furthermore, they quantified the time span during which abiotic tracer flux was obvious. But their study did not include $\delta^{13}\mathrm{C}$ of soil CO_2 efflux derived from their simulation. Thus, a quantitative interpretation of their observational soil efflux data cannot be inferred. However, simulation of $\delta^{13}\mathrm{C}_{\mathrm{efflux}}$ was provided in a very recent work (Ohlsson, 2011).

To avoid ambiguity, we reworded the text as follows and included the study of Ohlsson in the revised manuscript: “Recently, Ohlsson (2011) investigated $\delta^{13}C_{efflux}$ in the dataset of Subke et al. with a diffusion model, which was designed to simulate pulse labelling experiments. To our knowledge, this is the only study quantifying the effects of tracer application and associated changes in $\delta^{13}C$ in soil pore CO_2 on $\delta^{13}C_{efflux}$ in a mechanistic way.”

Reviewer #1: Page 86, Line 3. What precisely do you mean by enhance here? Be more specific with your word choice.

Author reply: We replaced “transients in dissolved CO_2 will likely lead to an enhancement of the abiotic tracer flux” by “transient conditions in dissolved CO_2 will likely increase the abiotic tracer flux compared to conditions where dissolution in water is not important”.

Reviewer #1: Page 86, Lines 11–12. Bowling et al. (2009) is not in soil, but rather in snow over soil – a potentially large difference in dynamics will result. There have not been many studies that address advection and $\delta^{13}CO_2$ although these two come to mind: Camarda et al., 2007; Kayler et al., 2010b.

Author reply: Following the reviewers suggestion we changed the text as follows: “Camarda et al. (2007) and Kayler et al. (2010b) investigated $\delta^{13}C$ of CO_2 in soil air pores and $\delta^{13}C_{efflux}$ in advective-diffusive regimes. Bowling et al. (2009) illustrated that the $\delta^{13}C$ in CO_2 within a snowpack depends on the physical nature of the transport mechanism, an analogous dependency may occur for CO_2 in soil pores.”

Reviewer #1: Page 86, Line 22. Which disequilibrium effect? You have outlined several before this and it is difficult to gauge what will be discussed in the following paragraph.

Author reply: Here we refer to disequilibrium effects in general, in particular all disequilibrium effects mentioned before. In the revised manuscript, we clarify this by replacing “The disequilbirium effect” by “Disequilibrium effects”.

Reviewer #1: Page 87, Line 2. I would include Koehler et al. 2010 here.

Author reply: The publication suggested by the reviewer is included in the revised manuscript as follows: “Some complications in the interpretation of diffusive flux profiles were discussed by Koehler et al. (2010).”

Reviewer #1: Page 87, Line 5. There have been several field and experimental papers that you should include in your discussion.

Author reply: We included field and laboratory investigations by Kayler et al. in the revised manuscript. Thus, we added the following text: "From a diffusion experiment involving artificial soil and CO_2 source, Kayler et al. (2008) concluded that non-steady-state effects must be considered in field investigations of $\delta^{13}C_{Rs}$ in soils. Furthermore, Kayler et al. (2010b) demonstrated in a field study the interrelation between perturbations of CO_2 in soil pores and aboveground measurement techniques for soil respiration."

Reviewer #1: Page 87, Lines 13–15. The sentence construction of this sentence needs improvement.

Author reply: In the revised manuscript, we clarified the sentence. Thus, the original sentence "These effects are expected to be even larger when additionally CO_2 dissolved in soil water is involved in soil-atmosphere CO_2 transport" was replaced by "The return to equilibrium takes longer if CO_2 in soil air pores exchanges with CO_2 dissolved in soil water. Accordingly, for a given sampling scheme with fixed sampling times (e.g. Keeling plots), the system deviates stronger from equilibrium when dissolved CO_2 is involved in soil gas transport. Thus, the divergence of $\delta^{13}C_{\text{efflux}}$ from $\delta^{13}C_{Rs}$ captured by sampling is expected to be even larger than predicted by Nickerson and Risk."

Reviewer #1: Page 87, Lines 16–29. This paragraph needs a thorough makeover. It is not clear at all what you are doing. Only by reading the article through and this paragraph several times over could I make sense of this paragraph. You should also specify at this time which dataset you use as the "true" respiratory signal.

Author reply: We revised the paragraph and split it in two. First, we introduce the experiment, outline the different measurements, and define which of them we use as "true" $\delta^{13}C_{Rs}$. In the second part, we then explain how we investigated the experimental data with our mechanistic model. The text now reads as follows:

"Here, we investigate the disequilibrium effect in ecosystem respiration in a field labelling experiment. In that experiment, a grassland ecosystem was exposed during daytime to CO_2 with a $\delta^{13}C$ of $-46.9‰$ for 2 weeks (Gamnitzer et al., 2009). Nocturnal $\delta^{13}C_{\text{efflux}}$ of the ecosystem was measured with three independent methods: steady-state open chambers, closed chambers (both in situ in the field), and laboratory-based cuvettes with excised soil+vegetation blocks. The $\delta^{13}C_{\text{efflux}}$ data of the open chamber measurements agreed with those of the cuvette measurements (Gamnitzer et al., 2009). This indicated that the $\delta^{13}C_{\text{efflux}}$ of open chamber measurements gave an accurate estimate of $\delta^{13}C_{Rs}$. In consequence, we used the open chamber data as 'true' $\delta^{13}C_{Rs}$ in the following.

The closed chamber measurements employed a Keeling plot approach. These estimates of ecosystem $\delta^{13}C_{\mathrm{efflux}}$ deviated by ~10‰ from $\delta^{13}C_{\mathrm{Rs}}$. We suspected that this discrepancy was associated with a disequilibrium effect. Thus, the aim of the present work is to quantify the impact of mechanisms which could underlie such a disequilibrium effect between $\delta^{13}C_{\mathrm{Rs}}$ and $\delta^{13}C_{\mathrm{efflux}}$. Among these mechanisms are diffusion of CO_2 in soil gas, dissolution of CO_2 in soil water and advection of soil gas. For this purpose, we present a new soil CO_2 transport model which accounts for respiratory CO_2 production, diffusion, dissolution, and advection of both $^{12}CO_2$ and $^{13}CO_2$. We applied the soil CO_2 transport model to evaluate mechanism(s) underlying abiotically-driven flux of tracer. We simulated the labelling experiment and predicted Keeling plot intercepts for nocturnal CO_2 accumulation in the closed chambers with the model. Simulation results were compared to observations to assess the quantitative importance of the different mechanisms underlying the disequilibrium effect. Lastly, we discuss the consequences of these mechanisms for commonly used isotopic approaches for the study of soil and ecosystem respiration."

Reviewer #1: Page 87, Lines 16–18. I think you want to specify you are only investigating these three phenomena. The way this sentence currently reads is that the disequilibrium is attributed only to these phenomena which I don't think is what you are trying to convey.

Author reply: We agree with the reviewer, we only investigated the effect of the three mechanisms on the disequilibrium effect. To clarify this in the manuscript text, we reworded the respective sentence: "Thus, the aim of the present work is to quantify the impact of mechanisms which could underlie such a disequilibrium effect between $\delta^{13}C_{\mathrm{Rs}}$ and $\delta^{13}C_{\mathrm{efflux}}$. In particular, we investigated effects of diffusion of CO_2 in soil gas, dissolution of CO_2 in soil water, and advection of soil gas due to chamber pressurization during labelling"

Reviewer #1: Page 87, Line 20. perhaps specify the data as ecosystem respiration data or as the isotopic signal of ecosystem respiration.

Author reply: We agree and revised the whole paragraph (see above).

Reviewer #1: Page 87, Line 22. It is not clear that you are referencing the older study and not the present one.

Author reply: We agree and revised the whole paragraph (see above).

Reviewer #1: Page 88, Line 12. Can you describe how shoot respiration is modeled in the equation set? It is not clear to me. Why don't you discuss the shoot dynamics at all in the paper? Are they just stable? If it is something you describe in the previous paper then mention it here (or better yet in the Introduction).

Author reply: In our model, shoot respiration is considered as respiratory CO_2 production above the soil. This is included in the revised manuscript as "P represents the total respiratory CO_2 production ($\mu mol\,m^{-3}\,s^{-1}$) of the system, including belowground and aboveground respiration".

Shoot dynamics were considered with respect to increasing tracer content during the labelling experiment. Details are explained in Section 2.3 "Simulation runs", see also below the reviewer comments regarding page 95, line 16 and line 26. Further shoot dynamics, in particular diurnal cycles, were not relevant for our investigation, since the samples contained in each Keeling plot were collected within a 12 min measurement cycle. Thus shoot dynamics other than increasing tracer content were not included in our model. We state this limitation of our model in the revised manuscript where appropriate (end of Section 2.1 "Soil CO_2 transport model"), "While the CO_2 production rate was set constant with time, the $\delta^{13}C_{Rs}$ was adjusted to changing tracer content for simulations of the labelling experiment (see Section 2.3.2 below)."

Reviewer #1: Page 90, Line 1. Please add the fractionation factor for each process you model.

Author reply: Values for the fractionations were added in the revision: "Fractionation for the dissolution of CO_2 in water was included according to Mook et al. (1974) and Vogel et al. (1970), with $H_2CO_3(aq)$ depleted compared to $CO_2(g)$ by $(-373/T + 0.19)$‰ and HCO_3^- enriched compared to $H_2CO_3(aq)$ by $(9866/T - 24.12)$‰, see Mook (2000)."

Reviewer #1: Page 90, Line 7. Define "Volume flux" – how can a volume have a flux?

Author reply: We replaced "volume flux of air per unit soil area" by the term "Darcy velocity".

Reviewer #1: Page 90, Line 11. Please justify the use of this model. As described above, you should also include other possible models to see how it impacts estimate of CO_2 diffusivity.

Author reply: As suggested by the reviewer, we included a range of diffusivity models: "Further estimates of (effective) soil diffusivity (Millington and Quirk, 1960; Moldrup et al., 1997, 1999, 2000, 2004) were used to investigate sensitivity to the choice of a D_{soil} model".

Please see also the Results and Discussion sections, were sensitivity of our model results to the choice of a D_{soil} model is shown and discussed.

Reviewer #1: Page 90, Line 20. What value(s) did you use for n?

Author reply: In this section (Section 2.1 "Soil CO_2 transport model") we describe the model in general. Parameter values used in the simulation runs are given in Section 2.3 "Simulation runs": "The soil of depth L=25 cm was divided into n=125 layers of thickness Δz=2 mm."

Reviewer #1: Page 91, Line 1. Do you describe the soil somewhere?

Author reply: In Section 2.2 "Field labelling experiment" we added a description of the soil: "The soil at the experimental site was mineral soil (inceptisols), which was used as arable land for more than 40 years before conversion to grassland in 1999 (Schnyder et al., 2006)."

Reviewer #1: Page 91, Line 5. Equation 11 needs to be displayed much better – it is a bit confusing the way it is currently stacked.

Author reply: We added a short description of the individual terms, e.g. "Dissolution", "Advective flow", and suggest the following layout:

$$\left(\varepsilon_\mathrm{a} + \underbrace{\varepsilon_\mathrm{w} K_\mathrm{H} RT\left(1+\frac{K_1}{[\mathrm{H}^+]}\right)}_{\text{Dissolution}}\right)\underbrace{\frac{c_\mathrm{a}(z,t+\Delta t)-c_\mathrm{a}(z,t)}{\Delta t}}_{\text{Conc. change with time}} \tag{11}$$

$$= \underbrace{D_\mathrm{soil}\frac{(c_\mathrm{a}(z+\Delta z,t)-c_\mathrm{a}(z,t))-(c_\mathrm{a}(z,t)-c_\mathrm{a}(z-\Delta z,t))}{\Delta z^2}}_{\text{Diffusive flow}} - \underbrace{v_\mathrm{Darcy}\frac{c_\mathrm{a}(z,t)-c_\mathrm{a}(z-\Delta z,t)}{\Delta z}}_{\text{Advective flow}} + \underbrace{P}_{\text{Resp.}}$$

Reviewer #1: Page 91, Line 11. What kind of impact on diffusion estimates do you expect from neglecting the gravel influence? The impact was substantial for Davidson et al., 2006. You should discuss this in the "discussion" section as well.

Author reply: Davidson et al. (2006) attributed <5% of CO_2 efflux to production in the C horizon. In contrast, we assumed that no CO_2 production occurred in the gravel layer. Since we attributed the entire CO_2 efflux to production in the soil layer, this corresponds to a shift in the depth distribution of the respiratory source. To account for this uncertainty, we tested the sensitivity of model results to shifts in this depth distribution. This information is added to Section 2.3.3 "Sensitivity analysis and model assumptions" of the revised manuscript:

"Disregarding respiratory CO_2 production in the gravel below the soil implied that the entire production of the observed CO_2 efflux was partitioned to the soil layer. Since this corresponded to a shift in the depth distribution of the respiratory source, sensitivity of the model results to variations in the depth distribution were investigated."

Reviewer #1: Page 91, Line 17. Reiterate that you are measuring ecosystem respiration

Author reply: We changed the text accordingly: "The temperate grassland ecosystem was continuously labelled for 2 weeks, and ecosystem respiration was measured every night."

Reviewer #1: Page 92, Lines 2–3. Why did you measure concentration and isotope ratio on separate instruments? What kind of error do expect from that – especially if you use this data for Keeling plots. You should definitely have a full analysis of the error in your estimates. Perhaps the range in error is negligible since you are labeling but please discuss this in your revision.

Author reply: In fact, it is possible to derive CO_2 mole fractions from the mass spectrometer measurements, since the recorded peak area is proportional to CO_2 mole fraction. Since the mass spectrometer was not calibrated for mole fraction measurements, we used an additional instrument for mole fraction analysis. However, the Keeling plots were derived from mass spectrometer measurements only, by relating δ^{13}C to the inverse of the peak area. Knowledge of the (constant) factor relating peak area and CO_2 mole fraction is not required for the Keeling plot intercept. This is stated more precisely, and measurement error is added, in the revised manuscript: "The CO_2 mole fraction and δ^{13}C were analysed in the field with an infrared gas analyser (LI 7000; . . .) and a continuous-flow isotope-ratio mass spectrometer (Delta Plus Advantage; . . .) (Schnyder et al., 2004). To ensure synchronous analysis of both quantities for the Keeling plots (see Section 2.2.2 below), CO_2 mole fraction was substituted by CO_2 peak area for the Keeling plots. Schnyder et al. (2004) demonstrated a proportional relationship between CO_2 mole fraction and CO_2 peak area. Measurement uncertainty of the mass spectrometer (SD of replicate measurements) was 0.09‰ for δ^{13}C, and corresponded to $\sim$2 µmol mol^{-1} for the CO_2 peak area."

As suggested by the reviewer, we included error analysis for the Keeling plot measurements in the Results section of the revised manuscript: "SD of the Keeling plot intercepts (parameter of linear fit) was 0.86‰ on average for an individual Keeling plot, with R^2=0.989 and CO_2 mole fraction covering a range of 120 µmol mol^{-1}. Potential biases due to choice of regression method (ordinary least squares regression vs. geometric mean regression) and mixing model approach (Keeling vs. Miller-Tans) were recently discussed (Pataki et al., 2003; Zobitz et al., 2006; Kayler et al., 2010a). Here, the average deviations were 0.09‰ between regression methods and 0.07‰ between mixing models approaches. In contrast, SD between Keeling plots in replicate ecosystem plots was 4.4‰."

Reviewer #1: Page 92, Line 11. Perhaps a brief description of the chambers is necessary, explicitly stating that the chamber is opened to the atmosphere, hence, the name "open top".

Author reply: We added a short description of the chamber system when we introduce the chambers in Section 2.2: "…an open-top chamber system was used, where the chambers were open at their top to the atmosphere ("open-top chambers"), and flushed with air."

Reviewer #1: Page 93, Line 11. Isn't 12 minutes a fairly long time for a chamber measurement? In the discussion you should describe how the time of measurement will impact the degree of fractionation by each mechanism you test.

Author reply: As mentioned by the reviewer, the timing of a measurement will impact the observed magnitude of the disequilibrium effect. This is explained as follows in the revised manuscript. The size of this impact depends on the actual site conditions, and is therefore not included in the generalised discussion.

"However, the time course of disequilibrium effects must be considered, since the disequilibrium is largest immediately after its initiation (the atmospheric change) and decreases with time (see Fig. 4). Thus, early initiation of sampling and short sampling duration will increase disequilibrium effects in a chamber measurement. In our grassland experiment, the disequilibrium effect was relevant for hours to days. This was consistent with observations in a boreal forest ecosystem, where the disequilibrium ('abiotic') tracer flux was significant for 48 h (Subke et al., 2009)."

The accumulation of CO_2 in a closed chamber headspace is known to influence diffusive soil CO_2 efflux. Nickerson and Risk (2009b) predicted that the associated deviation of Keeling plots from linearity increases with increasing chamber deployment time. In our experiment, the disequilibrium effect resulting from Keeling plot non-linearity (0.05‰) was negligible compared to the observed disequilibrium effect (11.2‰). This is included in the Results section of the manuscript as follows: "The magnitude of the disequilibrium effect resulting from Keeling plot non-linearity was derived from simulations …These Keeling plots yielded disequilibrium effects smaller than 0.05‰."

Reviewer #1: Page 93, Line 12. Calculate the turnover time of the chamber headspace.

Author reply: As suggested, we added the turnover time: "Sample air was pumped continuously from the chamber headspace to the analysers at $\sim 1.5\,\mathrm{L\,min^{-1}}$ at standard conditions (corresponding to a turnover time of 7.3 h for the chamber headspace air)."

Reviewer #1: Page 93, Lines 12–15. Isn't this a leak? How can you be sure that the isotopic signal of the CO_2 entering the chamber from the leak is the same as the surrounding atmosphere? Did you measure it? Couldn't this be a diffusive-advective flux into the chamber – will it affect the isotopic composition? You should state your assumptions accordingly.

Author reply: Yes, this is a leak. For the air entering the chamber via the leak we assumed advective flux: "The air removed for sampling was replaced by ambient air entering the chamber through an opening of 1–2 cm diameter. Assuming advective flow through this opening, the replacement air had the same mole fraction and δ^{13}C of CO_2 as the chamber headspace air at chamber closure."

Since CO_2 concentration in the chamber increased above ambient during the measurement cycle, diffusive CO_2 flux is opposing advective flux. We estimated that the advective flux of CO_2 into the chamber is at least two orders of magnitude larger than the diffusive flux out of the chamber. Thus, neglecting the fractionation associated with diffusive flux seems justified. This is consistent with the fact that no discrepancy between open chamber and closed chamber measurements was observed in the unlabelled ecosystem (control measurements, see Results 3.1 "Experimental tracer time series"). If fractionation due to diffusion had a significant impact on closed chamber measurements, then this would also be obvious in the control measurements (with the closed chamber Keeling plot intercept enriched compared to open chamber efflux measurements).

Reviewer #1: Page 93, Line 23. Pataki et al., 2003 is a better reference for terrestrial ecosystem research. Which regression did you use? Why did you use the Keeling plot versus Miller-Tans? How did you quantify the error in your measurement? There is plenty of research (Ohlsson et al., 2010; Zobitz et al., 2006; Kayler et al., 2010a, Nickerson and Risk, 2009) that discusses mixing model theory and application, please justify your model choice.

Author reply: Following the reviewer's suggestion, we included the Pataki et al. (2003) study, "The $\delta^{13}C_{\mathrm{efflux}}$ was determined with the Keeling plot approach (Keeling, 1958; see Pataki et al. (2003) for application to terrestrial ecosystem research)."

To account for the recent discussion on biased mixing model estimates, we calculated both GMR and OLS regressions, and compared Keeling plot and Miller-Tans approach. Deviations were within 0.1‰. We report this in the revised manuscript, Results section 3.1, as follows: "Potential biases due to choice of regression method (ordinary least squares regression vs. geometric mean regression) and mixing model approach (Keeling vs. Miller-Tans) were recently discussed (Pataki et al., 2003; Zobitz et al., 2006; Kayler et al., 2010a). Here, the average deviations were 0.09‰ between regression methods and 0.07‰ between mixing models approaches."

Reviewer #1: Page 94, Line 5. What do you mean advection was implemented? You mean it was modeled as … something or perhaps characterized by …? Again, I would stress here that you are investigating advection as a measurement artifact.

Author reply: To account for this reviewer suggestion, we reworded the corresponding phrase as follows: "During daytime labelling, a chamber pressurization of 5 Pa above ambient was observed due to high daytime air flow (Gamnitzer et al., 2009). This pressurization might have caused vertical (downwards) advection of soil air during daytime labelling. The impacts of this potential chamber artifact and of the dissolution of labelling CO_2 in soil water on the disequilibrium effect were investigated independently. For this purpose, model runs were performed including or excluding the individual mechanisms."

Reviewer #1: Page 95, Line 8. Do you really mean "replacing"? Or do the two end-members mix?

Author reply: We clarified this by replacing "Then closed chamber measurements of $\delta^{13}C_{\mathrm{efflux}}$ of the unlabelled ecosystem (control) were simulated by replacing the atmospheric layer with the chamber headspace volume, in which soil CO_2 efflux and shoot-respired CO_2 accumulated" by "Then closed chamber measurements of $\delta^{13}C_{\mathrm{efflux}}$ of the unlabelled ecosystem (control) were simulated. For closed chamber simulations, soil CO_2 efflux and shoot-respired CO_2 were mixed with ambient (background) air in the chamber headspace."

Reviewer #1: Page 95, Line 16. What exactly does "was adapted" mean here? Was it calculated somehow differently? Why did you have to do this?

Author reply: We clarified this as follows: "$\delta^{13}C_{\mathrm{Rs}}$ was adjusted to include a fractional contribution of labelled carbon according to the results of Gamnitzer et al. (2009) (see below for details)."

Reviewer #1: Page 95, Line 26 – Page 96, Line 2. You should include in your table of input parameters the different partitioning of carbon with time (I can only assume it changes over the day). Also, what did you use for the isotopic composition of these different sources? Does this matter for the model? I think this methods section is really incomplete. Additionally, how do these assumptions affect the fractionation partitioning, if at all?

Author reply: There seems to be a misunderstanding, which was clarified in the revision. Partitioning to the individual sources was constant with time, but the isotopic composition of the labelled (autotrophic) source changed during the labelling experiment. To make this clear to the reader, we added information on soil and shoot respiration (respiration rate and range of $\delta^{13}C_{Rs}$) to Table 1 and revised the respective paragraph:

"To account for the increasing amount of label in the respiratory source during the experiment, $\delta^{13}C_{Rs}$ was adjusted from day to day according to the 'true' time course of tracer. The latter was derived from the fit (Gamnitzer et al., 2009, see also Fig. 2, solid line) to the open chamber data (Fig. 2, open circles). To partition this signal into belowground (soil) and aboveground (shoot) respiratory CO_2 production (which are required as model input parameters), three respiratory sources were distinguished. The first, decomposition of soil organic matter, was located in the soil, did not respire any tracer ($\delta^{13}C$ constant at $-26.7‰$) and contributed 52% of ecosystem respiration (Gamnitzer et al., 2009). The other two sources reflected aboveground and belowground autotrophic respiration, where each was assumed to contribute 50% of autotrophic respiration. Both supplied recently-assimilated carbon from a labelled pool ($\delta^{13}C$ changed from $-26.7‰$ to $-64.4‰$ with a pool half-life of 2.6 d; Gamnitzer et al., 2009). In total, $\delta^{13}C_{Rs}$ changed from -26.7‰ to $-37.8‰$ for the belowground source and from $-26.7‰$ to $-63.5‰$ for the aboveground source in the simulation of the 14-days-long labelling period. In contrast, soil and shoot respiration rates were kept constant during a simulation run."

Reviewer #1: Page 96, Line 14. This information should not be in the results section but in the methods. You assume that this isotopic signal is the real value of $\delta^{13}C_{Rs}$ and you use it to compare with the "disequilibrium" data set.

Author reply: The respective information has been transferred to Materials and Methods, Section 2.3.2.

Reviewer #1: Page 97, Lines 4–5. What is the Bowling et al. study in snow referencing here?

Author reply: Bowling et al. (2009) discuss the mixing relationships of CO_2 mole fraction and $\delta^{13}C$ in a porous medium in detail. Their illustration facilitates understanding the mixing lines shown in the present manuscript. Thus, we suggest to leave this reference here and clarified the text: "The $\delta^{13}C$ profile corresponded to the theoretical mixing line (analogous to that illustrated by Bowling et al. (2009) for CO_2 in a snowpack) between atmospheric air ($-8.5‰$) and soil air ($-22.3‰$), . . ."

Reviewer #1: Page 98, Lines 18–19. Which mechanism are you discussing here? And, what is a "disequilibrium tracer flux"?

Author reply: The mechanism refers to the steps (1) to (6) described above in the manuscript. Furthermore, we specified "disequilibrium tracer flux" in the revised manuscript: "steps (1) to (6) acted as a tracer flux caused by soil-atmosphere isotopic disequilibria."

Reviewer #1: Page 98, Line 24. Use a more precise word than "attainment" – you are discussing a time delay.

Author reply: We replaced "attainment of the new steady-state" by "progression to the new steady-state".

Reviewer #1: Page 99, Line 24. This study does not provide direct evidence.

Author reply: This is considered in the revised manuscript, see "Main comments" above.

Reviewer #1: Finally, be consistent with your units of CO_2 concentration. You often use mole fraction to quantify CO_2 but you use different units in Fig. 3. Personally, I prefer $\mu\mathrm{mol\,mol^{-1}}$.

Author reply: Please note that there is a difference between concentration ($\mu\mathrm{mol\,m^{-3}}$ for molar concentration) and mole fraction ($\mu\mathrm{mol\,mol^{-1}}$). Unfortunately, these two terms are frequently mixed up. Here, we use both terms with respect to their actual meaning. Since CO_2 is commonly reported as mole fraction and this is independent of atmospheric pressure, we also present the data as mole fractions (denoted by C). However, for the soil CO_2 transport model it is much more convenient to provide the equations using concentration (denoted by c_a, c_w and c_T), e.g. diffusion is driven by the concentration gradient. To make this clear, we included the conversion between mole fraction and concentration in the revised manuscript: "Conversion between CO_2 concentration c_a ($\mu\mathrm{mol\,m^{-3}}$) and CO_2 mole fraction C ($\mu\mathrm{mol\,mol^{-1}}$) followed $c_\mathrm{a}=C/V_\mathrm{mol}$, where V_mol is the molar volume of an ideal gas ($22.4\,\mathrm{L\,mol^{-1}}$ at standard conditions; adapted to site conditions for temperature and pressure)."

Author reply to comments of Reviewer #2

Reviewer #2: Given the new understanding of NSS diffusion effects and their role in isotopic studies, it is certainly important to extend this understanding to a greater range of studies and ecosystems. In that respect, this is an important work. There are still too few studies where ecological researchers have indeed extended themselves into the arena of physical modeling, but it appears that more will likely have to in order to produce meaningful ecological results with good certainty. In this sense, many researchers will benefit from this study and others like it. I think the remarkable aspects of this particular effort include the water-phase diffusion kinetics, and the application to an interesting overall scenario which involves labeling, two types of chamber measurements, keeling plots, advection, etc. In that way, many of the individual transient impacts documented by authors are rolled together into this one study. The moisture diffusion results in particular are very striking, because they appreciably delay the onset of equilibrium in the model world. That in itself is, I think, the most important progress here. In the real world under time-varying conditions, it is likely (given water phase kinetics) that soils can never be at isotopic equilibrium! So, I think this effort is very useful.

Author reply: We thank the anonymous referee for reviewing our manuscript. The reviewer's concerns helped to improve the manuscript with respect to significance of the conclusions. In detail, we addressed his/her suggestions as follows.

Reviewer #2: The study isn't a demanding read, and is a straightforward approach to physical modeling of a measured system. The actual ecological processes aren't of real concern here – it is primarily the physical processes that are of interest. In that sense, I think the text is sufficient, useful, and appropriately spare. But, the results could more readily digested (and less skepticism generated) if there was more transparency related to aspects of modeling and model performance. Overall, my concerns reduce to two (the second in two parts):

1. The authors should recognize that a 1-D model like this is perhaps somewhat more limited relative to the other models they cite and which have been used by other authors. Many of these others are 3-D models, which allow transient disequilibria to develop more fully. The 1-D models deny lateral escape mechanisms to isotopologues, which would be a natural consequence of chambers footprints and super-ambient internal concentrations etc. So, the 1-D modeling approach used by the authors will probably underestimate the magnitude of transient fractionation. While a larger chamber minimizes edge effects, it still does not reduce entirely to a 1-D system. ACTION: The authors should provide related text in the methods and/or discussion section(s) to clarify these important differences - that results will not likely be exactly comparable to a 3-D model.

Author reply: Undoubtedly, a 1-D model is more limited than a 3-D model. In our application, the 1-D model sufficiently describes the relevant processes. In the revised manuscript, we argue more completely why we assume this. Hence, the text in Section 2.3.3 “Sensitivity analysis and model assumptions” now reads:

"In the present chamber investigation, a one-dimensional model was used. For the upper half of the soil layer, this simplification was appropriate due to mechanical suppression of lateral exchange by the chamber walls. The chambers were inserted into the soil via a soil collar to a depth of 12 cm, compared to a soil depth of 25 cm. Below the soil collar depth, lateral exchange processes were neglected according to the requirements provided by Nickerson and Risk (2009b,c) on soil diffusivity, air-filled porosity and chamber deployment time. Also the chamber used here was about 10 times larger in diameter than the one studied by Nickerson and Risk, minimizing edge effects. Furthermore, the influence of the atmospheric tracer on δ^{13}C in soil pores via gas exchange decreases with soil depth, suggesting that lateral effects were small below soil collar depth."

Reviewer #2: 2. The authors have the ability to reduce their exposure to methodological criticism by providing more transparency on model performance. There is lots of detail on the model's undercarriage, but not related to performance under known conditions or in the real world. I'd surely trade the latter for the former. In other words, I care less about what goes into a transient fractionation model (most of the equations are well known) than how well it does and how stable it is given normal ranges of uncertainty in input parameters. A great many of Reviewer 1's suggestions/concerns would fall into this category. Added information would allow the readers to more thoroughly evaluate the model's performance for themselves. Two types of related information are required:

a) Conformance to known conditions. Does the model generate the right results under known conditions (what are they), or compared to analytical solutions? The authors don't necessarily need to produce related results, but especially since this is a new (not previously used) model they should clearly outline what the validation metrics were, and whether they were met. There is related text (ie p94, line 10 related to impact of dissolution mechanism on model output), but it does not go far enough to say that results were as expected (with clear communication of those expectations). The authors have a good level of experience with this type of modeling so I'm not particularly worried that validation was not done – but it is a legitimate concern I think to say that model performance is somewhat of a black-box. The end result of the modeling exercise fits the data relatively well, and the authors should demonstrate that this is not coincidental. ACTION: The authors should provide related text in the methods section to clearly outline their expectations, the various synthetic tests they did to ensure that data was reasonable, and whether the model passed - either perfectly, or within acceptable limits.

Author reply: Model validation was conducted (1) by comparison of (numerical) model results with analytical solutions where analytical solutions were computable, and (2) by comparison to numerical results from an independent study which gives all necessary input parameters. Following the suggestion of the reviewer, we included the following information in the revised manuscript at the end of Section 2.1 "Soil CO_2 transport model":

"For model validation, analytical solutions of the mass balance equation (Eq. 1) were generated assuming steady-state conditions (no concentration change with time) and homogenous distribution of respiration with soil depth. For diffusive regimes, the analytical solution was derived according to Cerling (1984). For diffusive-advective regimes, the analytical solution was similar to that of Camarda et al. (2007) and Kayler et al. (2010b), as both groups studied diffusive-advective regimes with a gas reservoir beneath the soil instead of homogeneous production. For CO_2 mole fraction, numerical model results agreed within 0.2% with analytically-derived CO_2 mole fraction at all depths. Numerically-derived $\delta^{13}C$ was within 0.009‰ of analytically-derived $\delta^{13}C$. Furthermore, model estimates perfectly agreed with results presented by Cerling (1984) for the soil parameters given in that study."

Reviewer #2: b) Sensitivity to input parameters. While Table 1 provides some indication that sensitivity analyses were done, there are no related results. To what input parameters is the model sensitive? What's really important to get right in terms of parameterization data? That way, the authors could address concerns such as lacking pH measurements – for example do small errors in pH even matter? Or, do they matter a lot? I would have liked to see these results, particularly as model is "new", and also because the authors themselves opened the door to the issue of sensitivity (page 96, line 3) – which is a very important issue in modeling. The fact that the authors raised the issue suggests to me that they also have the results. Ideally, these would be the first of the results presented in the results section – to document the characteristics of the synthetic reality. ACTION: The authors should provide the results of sensitivity analyses, probably with text and/or table/figure.

Author reply: We agree with the reviewer that a sensitivity analysis of our model results strengthens our conclusions. Accordingly, we provide the related data in an additional figure and complemented the text in the Results section 3.4: "Sensitivity analysis (Fig. 7) shows that, within the uncertainties in model input parameters, simulations excluding dissolution did not reproduce the magnitude of the observed disequilibrium effect."

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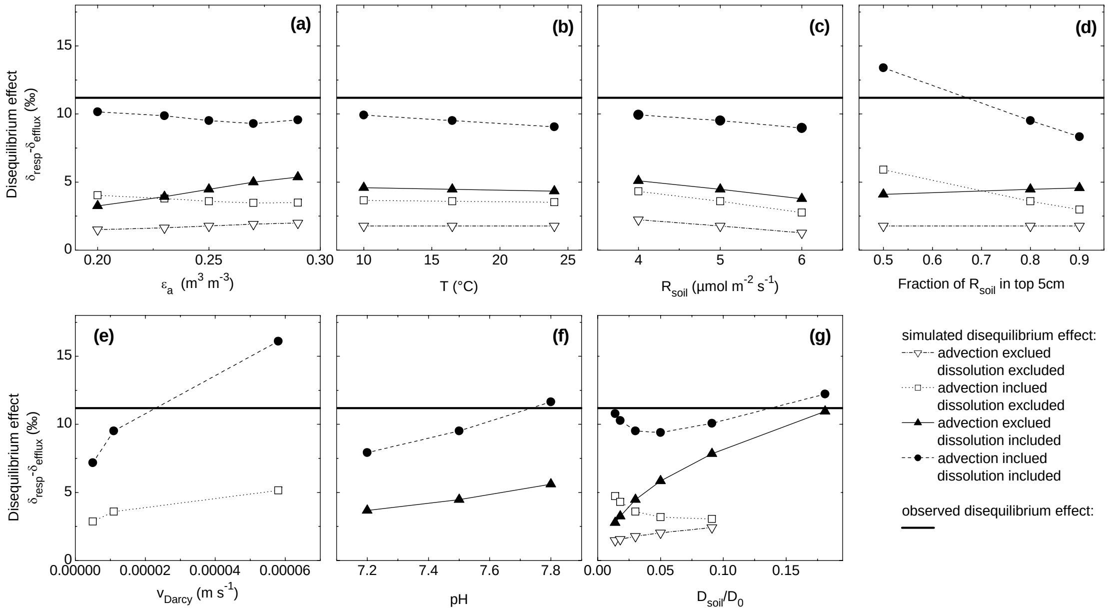

Figure 7: Sensitivity of simulated disequilibrium effect to variations in the model input parameters as shown in Table 1. Disequilibrium effects are shown as means during the labelling period. Simulations exclude (open symbols) or include (closed symbols) dissolution, and exclude (triangles) or include (circles and squares) advection. The observed disequilibrium effect is indicated by the thick solid line. The investigated parameters include (a) soil porosity, (b) soil temperature, (c) soil respiration rate, (d) depth distribution of soil respiration, (e) Darcy velocity, (f) pH, and (g) the choice of a soil diffusivity model.