Keeling plots are non-linear in non-steady state diffusive environments

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[1] End-member mixing models, such as the Keeling and Miller-Tans plots, are frequently used to interpret isotopic data collected from environments where mass transfer occurs due to diffusive processes, however, researchers do not commonly consider the effect of diffusive kinetic fractionation on assumptions of linearity in these mixing models. Risk and Kellman (2008) recently showed the potential for non-linearity in the Keeling plot approach, but their simplified model offers only a first order approximation of this effect in complex systems such as soils. Here we use 3-D numerical simulations and measurements of soil $^{13}$C-CO$_2$ flux accumulating in a static head space chamber to conclusively show that in diffusive environments Keeling plots are non-linear, violating key assumptions of the technique and potentially creating a large source of error in data analysis and interpretation. Citation: Nickerson, N., and D. Risk (2009), Keeling plots are non-linear in non-steady state diffusive environments, Geophys. Res. Lett., 36, L08401, doi:10.1029/2008GL036945.

1. Introduction

[2] The stable isotopic composition of carbon dioxide (CO$_2$) is a useful tool in understanding above and below ground carbon cycling processes and their response to changes in local and global environmental conditions [Andrews et al., 1999; Cerling et al., 1991; Ekblad and Högberg, 2000; Formanek and Ambus, 2004]. Linear end-member mixing models such as the Keeling and Miller-Tans plots are frequently used to interpret these types of isotopic data, collected from environments where mass transfer may occur due to turbulent and/or diffusive processes [Bowling et al., 2008; Buchmann et al., 1998; Zobitz et al., 2006]. While the applicability of these mixing models has been well proven in situations where bulk flow (i.e., turbulent mixing) dominates, researchers do not commonly consider the effect of differential rates of isotopologue transport (kinetic fractionation) caused by diffusive processes [Cerling et al., 1991; Amundson et al., 1998].

[3] Recent work by Risk and Kellman [2008] conducted under diffusive conditions has provided both experimental and theoretical evidence suggesting that kinetic fractionation creates non-linearities, violating the primary assumptions of these mixing models. Using static chamber collection of soil $^{13}$C-CO$_2$ surface flux, the authors showed that differential rates of isotopologue transport significantly affected the shape of $^{13}$C-CO$_2$ equilibration curves, creating non-linearity in Keeling plot data. Despite having experimentally validated their model predictions, Risk and Kellman’s [2008] simple 1-D two-box modeling approach offers only a first order approximation of the potential for non-linearity in Keeling plot data collected under diffusive regimes. Deficiencies in this important but simplistic model include: 1) Assumption of an unrealistic constant concentration bottom boundary condition that could potentially create considerably higher source fractionation [Davidson, 1995] than the expected $-4.4\%$ fractionation commonly observed in real soil systems [Cerling et al., 1991; Risk and Kellman, 2008; Bowling et al., 2008], 2) No chamber-to-soil feedback that disturbs natural steady state soil [CO$_2$] and $^{13}$C profiles leading to non-steady state isotopic fractionation, and 3) No means of simulating the effects of lateral diffusion of CO$_2$ away from the static chamber soil collar [Livingston et al., 2005, 2006] that in real situations could potentially enhance or dampen mixing non-linearities.

[4] In combination with statistical errors inherent in linear regression and extrapolation procedures [Pataki et al., 2003; Zobitz et al., 2006], application of linear models to non-linear data may yield results that are not representative of the biological processes they are meant to help understand. A more detailed understanding of these processes under realistic diffusive conditions would facilitate development of measurement and analysis tools appropriate for field deployment. In this study, we use more realistic 1-D and 3-D numerical simulations of soil CO$_2$ flux accumulating in a static head space chamber to conclusively show that Keeling plots are non-linear in diffusive environments. We validate model results using controlled laboratory experiments where chamber head space CO$_2$ concentration and $^{13}$C signatures are closely monitored. Finally, we provide estimates on the ranges of error when linear regression is inappropriately applied in diffusive environments, and discuss alternatives to the Keeling plot methodology.

2. Methods

2.1. Numerical Model

[5] To analyze the theoretical linearity of the Keeling plot we use a numerical isotopologue model that includes diffusion of gases in three dimensions. The isotopic composition of CO$_2$ is simulated by treating $^{12}$CO$_2$ and $^{13}$CO$_2$ as separate diffusing gases with isotopologue specific concentrations and diffusion rates ($^{12}$D = 1.0044 $^{13}$D) and by running one model iteration for each species. Model geometry is cylindrical and consists of 10 vertical and 5 radial soil layers, all of which produce gas in equal quantities ($\mu$mol m$^{-3}$ s$^{-1}$) and exchange gas with their nearest neighbor layers via Fick’s law of diffusion:

$$F_{ij} = -D_{ij} \frac{\Delta C_{ij}}{\Delta z_{ij}}$$

(1)

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analysis of 1-D and 3-D diffusion scenarios, we simulated a wide range of soil conditions with production rates ranging from 0.1–10 μmol m⁻² s⁻¹ and diffusivity varying between 0.005 to 3.8 × 10⁻⁶ m² s⁻¹. Biologically produced CO₂ was fixed at an isotopic signature of −25‰ for all synthetic scenarios. Finally, 3-D chamber simulations also included short (2.5 cm), medium (12.5 cm) and long (25 cm) soil collar scenarios.

2.2. Experimental Setup

[8] While a modeling approach is most appropriate to analyze the theoretical linearity of the Keeling plot, experimental confirmation of model validity is crucial. To validate theoretical predictions, we used data collected by Risk and Kellman [2008] during static chamber experiments that were carried out using triplicate soil incubation plots. Soils were a mix of commercial organic topsoil and silica sand, compacted to a bulk density of 1.24 kg L⁻¹. Plots were held at 15°C (average respiration rate of 1.15 μmol m⁻² s⁻¹) and 27% VWC inside growth chambers (Acadia University), which were maintained at 95% humidity. Atmospheric CO₂ concentrations and δ¹³C signatures were stable at 365 ppm and −9.8‰, respectively.

[9] A static chamber, similar in design to Ekblad and Högberg [2000], was used in conjunction with a 2.5 cm deep soil collar (head space volume w/collar = 3.93 × 10⁻⁴ m³) to measure δ¹³C-CO₂ emissions. Each chamber was deployed on a given soil plot for 0 to 720 minutes and sampled once at the end of the deployment period to minimize the effects of drawing air from the head space [Ohlsson et al., 2005]. These were replicated twice on each of the three plots for a total of 60 individual measurements.

3. Results

3.1. Linearity Under 1-D Diffusion

[10] Figure 1a shows simulated characteristic time series evolution for both chamber CO₂ concentration and δ¹³C signature for a production rate of 1 μmol m⁻² s⁻¹ and diffusivities ranging from 0.0466 to 1.81 × 10⁻⁶ m² s⁻¹. Despite the fact that these 1-D chamber simulations approach the source signature given sufficient time, the resulting Keeling plots (Figure 1b) show distinct non-linearities made more evident by their derivatives (dashed lines).

[11] For 1-D simulations, Keeling plot non-linearities were found to become more pronounced as diffusivity rates increased as a result of increased chamber-to-soil diffusive feedback. In all cases the resulting Keeling plot intercepts (δ₀, Figure 1b), which should reflect the isotopic composition of respiration, were skewed toward higher values as a result of the concave up curvature of the Keeling plot data.

3.2. Linearity Under 3-D Diffusion

[12] Simulations in 3-D show that lateral effects caused by diffusion of CO₂ around soil collars are negligible for all simulated soil collar lengths when the chamber-to-soil feedback is minimal - that is, when diffusivity and air-filled porosity are low. When diffusivities increase, the potential for chamber-to-soil feedback increases, as does the effect of lateral diffusion of soil gases around the collar, with the greatest deviations occurring when the soil collar is short. This lateral diffusion results in lower (compared to the 1-D
3.3. Experimental Results

Bottom of the soil collar. 3-D (2.5 cm collar only) cases were analyzed to estimate chamber head space CO2 concentration and soil collars. Figure 3 shows the experimental time series from experiments using a static chamber and short (2.5 cm) collars. Values in the Keeling plots for δ0 represent the y-intercept of the best linear RMS fit of all data.

Figure 2. (a) Simulated time series evolution for CO2 concentration (dashed lines) and δ13C signature (solid lines) for three different soil collar lengths (ξ) at moderate diffusivity and a production rate of 0.2 μmol m−2 s−1. (b) Resulting Keeling plots (solid lines) and two-point derivatives (dashed lines). Values in the Keeling plots for δ0 represent the y-intercept of the best linear RMS fit of all data.

Keeling plots for lateral diffusion cases show stronger concave up curvature than their 1-D counterparts (Figure 2b). In these cases the increased curvature is likely due to a combination of chamber-to-soil feedback mechanisms and lateral diffusion of CO2 around the soil collar. This lateral diffusion causes eventual equilibration of the chamber with soil CO2 concentrations and higher (because of atmospheric invasion effects) soil δ13C signatures at the bottom of the soil collar.

3.3. Experimental Results

To validate our simulated results we employed data from experiments using a static chamber and short (2.5 cm) soil collars. Figure 3 shows the experimental time series of chamber head space CO2 concentration and δ13C signature during the 12 hour chamber deployment periods. Each point represents the average of six δ13C-CO2 measurements and error bars show standard deviations (1 σ) of variability between three plots with replicate measurements. Using measured soil parameters and iteratively fitted values of soil diffusivity we attempted to reproduce the experimental results using the numerical model. As we expected, the 1-D model was unable to accurately predict CO2 concentration and δ13C signature evolution but our 3-D model (collar at 2.5 cm) was able to predict both time series (r2[CO2] = 0.95; r2[δ13C] = 0.93).

3.4. Non-linear Error Estimates

All model-generated Keeling plots for the 1-D and 3-D (2.5 cm collar only) cases were analyzed to estimate the potential error due to misapplication of linear models. Chamber deployment periods (DP) ranging from 20 minutes to 2 hours were considered in this analysis. Additionally, to allow direct comparison with our data we choose a subset of 5 samples with four evenly spaced over the first 12 minutes of deployment and the last sample taken at the end of the DP.

Table 1 presents the range of least squares linear regression estimates for δ0 and the maximum error from the actual value of −25‰ for all cases. High diffusivity soils with short (2.5 cm) soil collars yield the maximum potential errors in all simulations with the maximum observed error being 4.06‰ higher than the expected value of −25‰.

4. Discussion

Risk and Kellman’s [2008] constant bottom boundary assumption is an oversimplification that can lead to illegitimate correspondence between modeled and measured data. Where realistic in-soil δ13C-CO2 feedbacks are present in 1-D, the evolution of δ13C-CO2 signatures in the chambers (Figure 3, grey lines) is appreciably different than predicted by Risk and Kellman [2008]. This experimental data does, however, provide a situation where their simplified model can seem valid. With strong 3-D diffusion (short collars, short chambers, long deployment times) CO2 flows largely around the chamber, resulting in an effective constant concentration in the underlying soils. Ironically the ability of the 1-D Risk model to assimilate δ13C-CO2 feedbacks are present in 1-D, the evolution of δ13C-CO2 signatures in the chambers (Figure 3, grey lines) is appreciably different than predicted by Risk and Kellman [2008]. This experimental data does, however, provide a situation where their simplified model can seem valid. With strong 3-D diffusion (short collars, short chambers, long deployment times) CO2 flows largely around the chamber, resulting in an effective constant concentration in the underlying soils. Ironically the ability of the 1-D Risk

Table 1. Summary of Errors Induced by Application of Linear Regression (OLS) to Non-linear Mixing Data

<table>
<thead>
<tr>
<th>Dimension</th>
<th>DP (min)</th>
<th>δ0 Range (%)</th>
<th>Maximum Error (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-D</td>
<td>20</td>
<td>−24.99 → −24.13</td>
<td>+0.87</td>
</tr>
<tr>
<td>1-D</td>
<td>40</td>
<td>−24.98 → −23.90</td>
<td>+1.1</td>
</tr>
<tr>
<td>1-D</td>
<td>120</td>
<td>−24.96 → −23.57</td>
<td>+1.43</td>
</tr>
<tr>
<td>3-D</td>
<td>20</td>
<td>−25.01 → −22.95</td>
<td>+2.05</td>
</tr>
<tr>
<td>3-D</td>
<td>40</td>
<td>−24.99 → −21.86</td>
<td>+3.14</td>
</tr>
<tr>
<td>3-D</td>
<td>120</td>
<td>−24.98 → −20.94</td>
<td>+4.06</td>
</tr>
</tbody>
</table>

*Diffusivity and production rates included in the δ0 estimates vary from 0.005 to 3.8 × 10−6 m² s−1 and from 0.1 to 10 μmol m−2 s−1, respectively. Minimum errors were observed in low diffusivity, high production soils whereas maximum errors were observed in high diffusivity, low production soils.
and Kellman [2008] model to describe real data will weaken as the diffusion regime approaches 1-D. We suggest that constant concentration boundaries not be used for modeling isotopic transport in soils.

[18] Given our improved understanding of static chamber measurement of $\delta^{13}$C-CO$_2$ and the multiple lines of evidence presented here, we find that Keeling plot non-linearity is a universal feature of all diffusive environments due to differential rates of isotopologue transport. Lateral diffusion exacerbated these Keeling plot non-linearities in our simulated and experimental static chamber data and caused equilibrium of chamber $\delta^{13}$C-CO$_2$ with soil $\delta^{13}$C-CO$_2$. Simulations suggest that the magnitude of non-linearities are a function of the theoretical diffusive fractionation factor (4.4% for CO$_2$), chamber height, and most importantly, variable soil parameters such as diffusivity, porosity and CO$_2$ production rate.

[19] For the limited range of parameters used in our simulations, we observed Keeling plot extrapolation errors as high as 4%. This is not, however, the maximum possible error in all cases as many field studies have used collars which are significantly shorter than 2.5 cm [i.e., Ekblad and Högborg, 2000]. Errors due to non-linearity were found to increase as sample time increased, sitting in direct conflict with the recommendation of using long sampling times to increase Keeling plot accuracy made by Ohlsson et al. [2005]. Errors resulting from non-linearity are, of course, compounded by known statistical errors associated with Keeling plot extrapolations [Pataki et al., 2003; Zobitz et al., 2006], ultimately leading to poor resolution of isotopic source signatures. These considerations are all particularly important when researchers are exploiting small differences in isotopic signature.

5. Solutions

[20] Ideally linear end-member mixing models, such as the Keeling plot, should not be applied in diffusive environments. For soil respiration work, several promising new hardware methodologies [Subke et al., 2004; Bertolini et al., 2006; Midwood et al., 2008; Mora and Raich, 2007] are under development which do not rely on mixing model approaches and potentially provide a more direct measurement of source isotopic composition. For chamber time-series data which has already been collected for analysis with Keeling plots, the model presented here provides a suitable alternative for determining actual source signatures. It requires detailed soil-specific information (CO$_2$ production rate and expected depth distribution, atmospheric $\delta^{13}$C-CO$_2$, diffusivity, chamber collar insertion depth, etc.) and may be computationally intensive if fitting of unknown parameters is required, but offers the most promise for interpretation of previously collected and new data.

6. Conclusions

[21] Isotopes are a critical tool in ecosystem research and to enhance data quality, more work needs to be done to assess the magnitude and extent of physical fractionations and the theoretical underpinnings of analytical techniques. We conclude that assumptions of the oft-used Keeling plot technique are violated in diffusive environments and that misapplication of this technique can give rise to significant error. Ideally the use of the Keeling plots in these environments should be discontinued in favor of more appropriate sampling and analytical techniques.

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