CO₂ Flux Estimation by Different Regression Methods from an Alpine Meadow on the Qinghai-Tibetan Plateau

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ABSTRACT

CO₂ efflux was estimated using different regression methods in static chamber observation from an alpine meadow on the Qinghai-Tibetan Plateau. The CO₂ efflux showed a seasonal pattern, with the maximum flux occurring in the middle of July. The temperature sensitivity of CO₂ efflux (Q₁₀) was 3.9, which was at the high end of the range of global values. CO₂ emissions calculated by linear and nonlinear regression were significantly different (p < 0.05). Compared with the linear regression, CO₂ emissions calculated by exponential regression and quadratic regression were 12.7% and 11.2% larger, respectively. However, there were no significant differences in temperature sensitivity values estimated by the three methods. In the entire growing season, the CO₂ efflux estimated by linear regression may be underestimated by up to 25% compared to the real CO₂ efflux. Consequently, great caution should be taken when using published flux data obtained by linear regression of static chamber observations to estimate the regional CO₂ flux in alpine meadows on the Qinghai-Tibetan Plateau.

Key words: CO₂ emission, static chamber technique, nonlinearity, underestimation, Qinghai-Tibetan Plateau


1. Introduction

Carbon dioxide (CO₂) plays an important role in climate change. Accurate estimation of the CO₂ flux between terrestrial ecosystems and the atmosphere is becoming an increasingly important component of researches on natural ecosystems (Rayment, 2000). The key point of accurate measurement of CO₂ flux is to measure the CO₂ without disturbing the diffusion gradients (Bain et al., 2005). Eddy covariance techniques are ideal to allow continuous measurement the CO₂ flux (Baldocchi et al., 1988). However, the technique is very expensive and it is very difficult to identify the specific area that may contribute to observed fluxes (Bain et al., 2005; Norman et al., 1997). Static chamber methodology is still widely used for measuring CO₂ fluxes in natural ecosystems particularly where a power supply is unavailable (Kutzbach et al., 2007). When this method is used, the change of gas concentration in the chamber over time usually is assumed to be linear and the flux is estimated by linear regression (Cao et al., 2004; Hu et al., 2008). However, a linear regression can underestimate the real flux because when a chamber is placed over the soil, the concentration gradient between the soil and the atmosphere is altered (Conen and Smith, 2000; Healy et al., 1996). Exponential (Nakano et al., 2004) or quadratic (Wagner et al., 1997) regression have been applied in flux
calculations, because these nonlinear models can correct the error caused by linear regression. To the best of our knowledge, no research has examined the CO$_2$ flux underestimation by static chamber methodology on the Qinghai-Tibetan Plateau.

The objective of this study is: (1) to quantify the seasonal variation of CO$_2$ emission and to study the effect of temperature on the CO$_2$ efflux; (2) to compare CO$_2$ emission by different of calculation methods and to correct the error due to the application of linear regression. This research can contribute to reducing the uncertainty in estimating the regional CO$_2$ flux over Qinghai-Tibetan plateau.

2. Materials and methods

2.1 Site description

Sampling was conducted in an alpine meadow located at the Haibei Alpine Meadow Ecosystem Research Station, Northwest Plateau Institute of Biology, Chinese Academy of Sciences (37°37′N, 101°12′E and at an altitude of 3250 m above sea level). The local climate is characterized by strong solar radiation with long, cold winters, and short, cool summers. The average annual air temperature was −1.7°C. The mean, maximum and minimum daily average air temperatures were 8.7°C, 15.6°C and 2.5°C, respectively, in summer and −13.2°C, −2.2°C, and −22.1°C, respectively, in winter. Annual mean precipitation is 580 mm, and about 80% of precipitation is concentrated in the growing season from May to September (Li et al., 2004). The soil is a clay loam with an average thickness of 65 cm. The soils, which are classified as Mat Cry-gelic Cambisols according to the Chinese national soil survey classification system (Chinese Soil Taxonomy Research Group, 1995), are wet and high in organic matter content. Characteristics of the soils in the experimental plots are listed in Table 1 (Zhang and Cao, 1999). The plant community in this alpine meadow is dominated mainly by Kobresia humulis, Stipa aliena, Elymus nutans, Saussurea coarsa, Gentiana straminea and Potentilla nivea (Wang et al., 1998).

### Table 1. Characteristics of the alpine meadow soil*

<table>
<thead>
<tr>
<th>Depth (cm)</th>
<th>Organic matter (%)</th>
<th>C/N</th>
<th>pH</th>
<th>CaCO$_3$ (%)</th>
<th>CEC (cmol kg$^{-1}$)</th>
<th>Total N (%)</th>
<th>Hydrolyzable N (mg kg$^{-1}$)</th>
<th>Total P (%)</th>
<th>Available P (mg kg$^{-1}$)</th>
<th>Total K (%)</th>
<th>Available K (mg kg$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0−10</td>
<td>12.07</td>
<td>16.7</td>
<td>7.3</td>
<td>0</td>
<td>29.88</td>
<td>0.419</td>
<td>104</td>
<td>0.090</td>
<td>7.3</td>
<td>2.14</td>
<td>315</td>
</tr>
<tr>
<td>10−20</td>
<td>8.64</td>
<td>12.7</td>
<td>7.7</td>
<td>0</td>
<td>31.02</td>
<td>0.392</td>
<td>107</td>
<td>0.088</td>
<td>3.6</td>
<td>2.08</td>
<td>187</td>
</tr>
<tr>
<td>20−50</td>
<td>3.09</td>
<td>10.4</td>
<td>8.3</td>
<td>4.29</td>
<td>16.44</td>
<td>0.173</td>
<td>79</td>
<td>0.080</td>
<td>0.2</td>
<td>2.18</td>
<td>97</td>
</tr>
<tr>
<td>50−70</td>
<td>1.29</td>
<td>11.9</td>
<td>8.5</td>
<td>4.75</td>
<td>14.94</td>
<td>0.063</td>
<td>35</td>
<td>0.063</td>
<td>7.5</td>
<td>2.14</td>
<td>71</td>
</tr>
<tr>
<td>70−110</td>
<td>3.54</td>
<td>11.3</td>
<td>8.4</td>
<td>6.44</td>
<td>25.32</td>
<td>0.181</td>
<td>59</td>
<td>0.069</td>
<td>1.6</td>
<td>-</td>
<td>116</td>
</tr>
</tbody>
</table>

* Data are cited from Zhang and Cao, 1999; “−” represented the data was not determined.

2.2 CO$_2$ fluxes and environmental parameters measurements

CO$_2$ fluxes were measured using an opaque, static, manual stainless steel chambers/gas chromatography technique (Wang and Wang, 2003). The chamber is an open-bottom a square box (dimensions 50×50×50 cm$^3$) equipped with a fan installed on the top wall to create turbulence when the chamber is closed. The outside of the chamber was covered with white plastic foam to reduce the impact of direct radiative heating during sampling. In April 2008, nine stainless steel bases (50×50×20 cm$^3$) with water grooves on top were installed in the alpine meadow. These stainless steel bases were laid out randomly. The CO$_2$ flux we measured was the ecosystem respiration including heterotrophic respiration by microbes and autotrophic respiration by plants. Gas samples were taken between 0900 and 1100 LST four times a month from May to October in 2008. The flux measured at this time is assumed to be the mean flux of the corresponding day. On a sampling day, nine chambers were placed over the bases filled with water in the groove to ensure air-tightness and the gas samples were taken simultaneously. Gas samples inside the chamber were taken every 10 min over a 30 min period by using 100 mL plastic syringes (for a total of four samples). Gas samples of CO$_2$ concentrations were analyzed with gas chromatography (HP Series 4890D, Hewlett Packard, USA) within 24 h following gas sampling. The gas chromatography was equipped with a flame ionization detector (FID) for CH$_4$ analysis. CO$_2$ was converted to CH$_4$ prior to analysis with the FID, using a Ni catalyst and H$_2$ under condition of 375°C. The gas chromatography configuration for analysis was according to the method of Wang and Wang (2003). The 5 cm soil depth temperature ($T_s$) and 10 cm depth volumetric soil moisture (%) were monitored at each chamber during gas sample collection. $T_s$(°C) was measured using a digital thermometer (SN2202, China). Volumetric soil moisture (%), m$^3$ H$_2$O m$^{-3}$ soil was determined using a time domain reflectometry (MP-Kit, China). The volumetric soil moisture was transformed to water-filled pore space (WFPS): WFPS=...
VSM/(1–BD/PD), VSM is volumetric soil moisture, BD is soil bulk density and PD is soil particle density (2.65 g cm$^{-3}$).

2.3 Methods for computation of CO$_2$ fluxes

2.3.1 Linear regression

\[ C_t = a_0 + a_1 t , \]

$C_t$ (µmol mol$^{-1}$) is the CO$_2$ concentration measured at time $t$ (min); $a_0$ (µmol mol$^{-1}$) and $a_1$ (µmol mol$^{-1}$ min$^{-1}$) are regression parameters. In this method, $\frac{dC}{dt}|_{t=0}$ is equal to $a_1$.

2.3.2 Quadratic regression

\[ C_t = b_0 + b_1 t + b_2 t^2 , \]

$b_0$ (µmol mol$^{-1}$), $b_1$ (µmol mol$^{-1}$ min$^{-1}$) and $b_2$ (µmol mol$^{-1}$ min$^{-2}$) are regression parameters. $b_1$ is $\frac{dC}{dt}|_{t=0}$, and $b_0$ represents the CO$_2$ concentration at $t=0$, while $b_2 t^2$ is considered as an extra loss term compared to the linear regression (Wagner et al., 1997).

2.3.3 Exponential regression

The exponential model of Nakano et al. (2004) was adopted, which is based on simplified diffusion theory.

\[ F = -D \frac{\partial C}{\partial z} . \]

According to Fick’s law of diffusion, the steady flux of gas $F$ (µmol m$^{-2}$ m min$^{-1}$) is determined by the soil-gas diffusivity $D$ (m$^2$ min$^{-1}$) and the vertical gradient of gas concentration $[\partial C/\partial z]$ (µmol m$^{-1}$ m$^{-1}$).

\[ F_i = D \frac{C_d - C_i}{d} . \]

At time=$t$, the flux ($F_i$) at the soil surface is determined by $C_i$ (µmol mol$^{-1}$), $C_d$ (µmol mol$^{-1}$), and $d$ (m). $C_i$ represents CO$_2$ concentration at the soil surface at time=$t$ and $C_d$ represents CO$_2$ concentration at an unknown depth $d$ where the CO$_2$ concentration is constant.

\[ F_i = h \frac{\partial C_a}{\partial t} . \]

The CO$_2$ exchange rate as estimated by the static chamber method is calculated as follows. $h$ (m) is the height of the chamber, and $C_a$ (µmol mol$^{-1}$) is the CO$_2$ concentration in the chamber. It is assumed that the CO$_2$ in the chamber is fully turbulent so that $C_a$ is equal to $C_t$. Combining (4) and (5):

\[ \frac{\partial C_t}{\partial t} = \frac{D}{hd} (C_d - C_t) . \]

Replacing $D/\partial$ with $k$ and computing the indefinite integral of (6) yield:

\[ C_t = A \exp(-kt) + C_d \]

(7)

Here, $A$ is the integral constant.

By fitting an exponential function (7) to the gas concentration changes in the chamber over times, the parameters $A$ and $k$ can be estimated. $\frac{dC}{dt}|_{t=0}=-Ak$.

\[ F' = 60h \frac{dC}{dt}|_{t=0} \frac{M_m}{V_m} . \]

(8)

The flux data we used $F'$ (mg CO$_2$ m$^{-2}$ h$^{-1}$) is transformed by (8). $M_m$ (g mol$^{-1}$) is molar weight of CO$_2$, and $V_m$ (m$^3$ mol$^{-1}$) is the molar volume, calculated based on air temperature and pressure.

2.4 Data processing

All regressions were calculated in the math calculation software MATLAB (Matrix Laboratory) (MathWorks, Inc., USA). Linear regression, quadratic regression and exponential regression were performed using the function of regress, polyfit and nlinfit, respectively. Therefore, we effectively have get three flux data sets with each set comprised of nine replicates. In the whole growing season, the van’t Hoff equation $[y = A \exp(BT)]$ (Zheng et al., 2009) was established to calculate the temperature sensitivity of CO$_2$ fluxes to the changes of 5 cm depth soil temperature ($T_s$). The terms $A$ and $B$ are the ecosystem respiration at 0°C and the temperature sensitivity coefficient, respectively. Every replicate from the three resulting flux data sets was employed to calculate $A$ and $B$, respectively. The temperature sensitivity of CO$_2$ efflux ($Q_{10}$) was calculated from the corresponding $B$ [$Q_{10}=\exp(10B)$]. SPSS13.0 was used to apply Tukey’s test to identify the differences among the three regression methods regarding CO$_2$ emission and the $A$ and $B$ values.

3. Result

3.1 Changes of CO$_2$ concentrations in the chambers

Typical changes of CO$_2$ concentration inside the chamber were plotted (Fig. 1a), based on sampling in August. CO$_2$ concentration inside the chamber gradually increased over time from ambient atmospheric levels (about 380 µmol mol$^{-1}$). The line fitted by the linear regression goes nearly directly through the four actual measurement points. The first and the fourth point were below the line, while the second and the third point were above the line. Because the exponen-
Fig. 1. (a) Example of CO$_2$ accumulation in a chamber and linear and exponential fits to the data. (a') The inset graph shows a magnification at $t=0$. (b) Slope variation of the two regression curves.

Fig. 2. Seasonal variations of (a) soil temperature and WFPS, (b) CO$_2$ fluxes calculated by different methods, and (c) cumulative CO$_2$ emissions estimated by different methods. The error bars indicate the standard errors of mean ($n=9$). The legends in (c) represent the same meaning as in (b).

3.2 Differences of CO$_2$ fluxes and temperature sensitivity calculated by the three regression methods

The seasonal variation of $T_s$, WFPS and CO$_2$ flux are shown in Fig. 2. $T_s$ gradually increased from the beginning of the growing season and reached maximum in the middle of July (Fig. 2a). The mean $T_s$ for the growing season was 9.7°C. The alpine meadow was a source of CO$_2$ and displayed a clear seasonal pattern. The magnitude of CO$_2$ flux increased very sharply in the middle of June and the maximum flux occurred in the middle of July (Fig. 2b). The magnitude of CO$_2$ flux estimated by the linear method ranged from 288 mg m$^{-2}$ h$^{-1}$ to 987 mg m$^{-2}$ h$^{-1}$, which was of the same order of magnitude in previous alpine meadow studies (Cao et al., 2004; Hu et al., 2008). Based on Tukey’s test, the CO$_2$ emission estimated by the nonlinear method was significant larger than that of linear method ($p < 0.05$) (Table 2). CO$_2$ emission calculated by exponential regression and quadratic regression were 12.7% and 11.2% greater than the level calculated by linear regression, respectively. However, the temperature sensitivity coefficients ($B$) calculated by the various methods were almost identical (Table 2).

4. Discussion

4.1 Nonlinear increasing of CO$_2$ concentration in static chamber

The linear coefficient of determination $R^2$ was often used in the close chamber data processing to quantify the data quality. In our study, the $R^2$ values of all the gas data linear regressions were greater than 0.95 (data not shown). However, the high values of $R^2$ are not an appropriate criteria by which to check the linearity of the fits and can not provide evidence
to exclude serious biases of the flux estimates by the linear regression (Huber, 2004; Hibbert, 2005). This is because $R^2$ is only a measurement of the explained variance normalized to the total variance (Kutzbach et al., 2007). Additionally, modeling studies have shown that the flux estimated by linear regression was at only 84% of the source strength even through the concentration increase in the chamber was almost linear ($R^2>0.99$) (Conen and Smith, 2000). This was because a considerable proportion of gas was stored in the soil profile after the chamber was deployed. The slope of the exponential curve was greater than that of a straight line at the beginning of chamber deployment ($t=0$) (Fig. 1a'), indicating that the linear regression underestimated the predeployment CO$_2$ flux. With the increase of chamber deployment time, the slope of the exponential curve gradually decreased and became smaller than that of the linear method at $t=30$ min (Fig. 1b). The nonlinear regression could describe the variation of CO$_2$ concentrations inside the non-steady state chamber more exactly than the linear regression.

4.2 Underestimation by linear regression in static chamber observation

Comparisons of regression methods for gas flux calculation have been made previously. Kroon et al. (2008) reported that cumulative estimates via linear regression method are 60% below the cumulative estimates from exponential regression. Hutchinson and Livingston (1993) reported that flux estimated by linear regression was 47% smaller than estimated by nonlinear model. Nakano et al. (2004) found that linear regression underestimated the flux by 158% to −2% compared to the nonlinear methods. The magnitude of difference was attributed to chamber height, deployment period and soil air-filled porosity (Livingston et al., 2005; Nakano et al., 2004).

The underestimation of real flux levels by linear regression has also been reported by many studies. The underestimation ranged from 10% (Rayment, 2000) to 40%–50% (Norman et al., 1997; Pumpanen et al., 2003, 2004). Based on numerical simulations of closed chamber experiments with closure times of 20 min, Matthias et al. (1978) found that exponential regression developed from simplified diffusion theory still underestimated the real fluxes by 11%. Compared with exponential regression, linear regression underestimated the CO$_2$ flux by 12.7% in our study (Table 2). Therefore, we deduce that linear regression underestimates the real fluxes by up to 25%, based on the calculation $[(1+12.7\%) \times (1+11\%) - 1] \times 100\% = 25\%$. This result was within the range reported by previous researches. Figure 3b also showed that the CO$_2$ estimates based on the exponential method were close to those obtained by the quadratic method, while the CO$_2$ estimates based on the exponential method were obviously greater than those calculated from the linear method, and furthermore the larger fluxes showed larger differences (Fig. 3a). This result was consistent with the analyses of Stolk et al. (2009) and Kroon et al. (2008).

The temperature sensitivity of respiration has studied intensely on the global scale and across China, across various kinds of ecosystems (Tjoelker et al., 2001; Peng et al., 2009; Zheng et al., 2009). Reported $Q_{10}$ values on global scale vary from 1.3 to 3.3 and have a median value of 2.4 (Raich and Schlesinger, 1992). Our result was 3.9, which was at the high end value of global scale, indicating that increasing temperature in the future will exert great impacts on the ecosystem respiration of the alpine meadow. The temperature sensitivity coefficients ($B$) estimated by different regression methods were almost identical, while the intercepts of these exponential curves on the y-axis showed relatively obvious differences (Table 2). The analysis above implied that the flux discrepancy between nonlinear and linear methods was a systematic error, and the effect of this error on calculations of CO$_2$ emission could not change with sample date.

4.3 Causes of nonlinearity

Firstly, the nonlinearity of chamber gas concentration was caused by changes of the concentration gradients between the soil and air in the headspace of the

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**Table 2. Comparison of cumulative CO$_2$ emission (g m$^{-2}$) and temperature sensitivity calculated based on different regression methods in the growing season.**

<table>
<thead>
<tr>
<th>Regression methods</th>
<th>Cumulative CO$_2$ emission Mean</th>
<th>Percent increase</th>
<th>van’t Hoff equation $y = A exp(B T_s)$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linear method</td>
<td>2090(75)$^a$</td>
<td>40%</td>
<td>$A = 155(12.1)^a$, $B = 0.136(0.005)^a$, $Q_{10} = 3.90$</td>
</tr>
<tr>
<td>Quadratic method</td>
<td>2325(62)$^b$</td>
<td>36%</td>
<td>$A = 171(12.3)^a$, $B = 0.137(0.005)^a$, $Q_{10} = 3.94$</td>
</tr>
<tr>
<td>Exponential method</td>
<td>2357(58)$^b$</td>
<td>34%</td>
<td>$A = 176(11.8)^a$, $B = 0.135(0.005)^a$, $Q_{10} = 3.86$</td>
</tr>
</tbody>
</table>

Note: $T_s$ is 5 cm depth soil temperature. Percent increase (%) of cumulative CO$_2$ emission calculated by nonlinear methods is compared with the value calculated by the linear method. Values with different superscript letters in the same column are significantly different ($p <0.05$) based on Tukey’s test. Values in parentheses are standard errors of the mean ($n=9$).
chamber. In other words, the measurement method itself alters the measurand (Kutzbach et al., 2007). This feedback can not be avoided, but its influence can be minimized by increasing chamber height and reducing closure period. Rochette and Eriksen-Hamel (2008) suggested that chamber height in combination with the closure period should larger than 40 cm h\(^{-1}\) (e.g. chamber height=20 cm; closure period=30 min). The methodology of gas sampling in our research followed this criterion. Secondly, gas leakage after chambers are deployed is an important cause of gas concentration nonlinearity in chamber. Leaking of gas could be from the chamber itself, which could be avoided by good airproof design in chamber manufacture. The gas can also escape under the chamber base (Stolk et al., 2009). Rochette and Eriksen-Hamel (2008) proposed that chamber base insertion would exert little influence on flux estimation if the insertion depth/deployment duration ratio is greater than 12 cm h\(^{-1}\). Macropore, like worm holes or shrinkage cracks in soil, are another way by which gas leaks from the chambers. Stolk et al. (2009) attributed the nonlinearity to gas leakage from the soil cracks caused by dry conditions. In our observation period, the soil moisture dramatically decreased in June (Fig. 2a). However, we did not find a difference of temperature sensitivity between the linear and nonlinear methods, which implies that the nonlinearity of gas concentration was not caused by soil drying.

### 4.4 Implications for future research

In order to minimize the disturbance effects on the measurement of real flux, researchers could design relatively tall chambers and reduce the deployment period of gas sampling (Davidson et al., 2002). However, such methods might reduce the sensitivity of the chamber system for measuring the small fluxes because of clear trade-offs that exist between accuracy and precision in determining gas fluxes using chamber methods (Venterea et al., 2009). Tunable diode laser spectrometers (TDLS) measurements can achieve high-precision analysis of CO\(_2\) concentration in the atmosphere (Bowling et al., 2003). With decreases in the equipment prices, these instruments might soon be widely applied in chamber method. In addition, with the development of time-dependent diffusion models applied in static chambers (Livingston et al., 2005; Liu and Si, 2009), one could evaluate the effect of the chamber on the gas exchange process more accurately. Lastly, in order to get more accurate flux data, the chamber system should be better calibrated using the diffusion box method (Widen and Lindroth, 2003; Pumpanen et al., 2003, 2004) or trace gas method (Kroon et al., 2008).

### 5. Conclusions

In conclusion, we estimated CO\(_2\) efflux using different regression methods with static chamber observations from an alpine meadow on the Qinghai-Tibetan Plateau. Compared with the linear regression, CO\(_2\) emission calculated by exponential regression and quadratic regression were 12.7% and 11.2% larger, respectively. However, there were no significant differences in temperature sensitivity values estimated by the three methods, indicating that the difference in flux between the nonlinear and linear method represents a systematic error. Over the entire growing season, the CO\(_2\) efflux estimated by linear regression may be underestimated by up to 25% compared to the real CO\(_2\) efflux. Consequently, great caution should be taken when using published flux data obtained by linear regression in static chamber observations to es-

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**Fig. 3.** Comparison of the CO\(_2\) fluxes calculated by the exponential method and (a) the linear method or (b) quadratic method. The solid line is the fitted linear regression line, which is forced through zero, and the regression equation and \(R^2\) are given. The dashed line indicates the 1:1 line.
timate the regional CO$_2$ flux for alpine meadows on the Qinghai-Tibetan Plateau.

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