

## NOTE

# Development of chamber-based sampling technique for determination of carbon stable isotope ratio of soil respired CO<sub>2</sub> and evaluation of influence of CO<sub>2</sub> enrichment in chamber headspace

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We developed an experimental method for precise determination of carbon stable isotope ratio ( $\delta^{13}\text{C}$ ) of soil-respired CO<sub>2</sub> under natural condition. We devised a flask sampling system optimized for collecting soil-respired CO<sub>2</sub> to minimize the measurement artifacts related to pressure anomaly. The  $\delta^{13}\text{C}$  of soil-respired CO<sub>2</sub> was estimated from relationship between change rates of the CO<sub>2</sub> mole fraction and the  $\delta^{13}\text{C}$  of the CO<sub>2</sub> in a closed chamber at the soil surface by using two end-member simple mixing model. We tested the influence of CO<sub>2</sub> enrichment in the soil-chamber headspace on the estimates of the  $\delta^{13}\text{C}$  of soil respired CO<sub>2</sub> by using high-precision measurements of CO<sub>2</sub> mole fraction and  $\delta^{13}\text{C}$ . To our results, the estimates of the  $\delta^{13}\text{C}$  of soil respired CO<sub>2</sub> was rather unsusceptible to the influence of the CO<sub>2</sub> enrichment in the chamber as compared with the soil CO<sub>2</sub> efflux. Improvement of analytical precision of  $\delta^{13}\text{C}$  is preferred approach to reduce the error in the estimates of  $\delta^{13}\text{C}$  of soil respired CO<sub>2</sub>. On the other hand, extending the sampling range of CO<sub>2</sub> mole fraction in the chamber can be cost-effective means for the error-reduction practically.

Keywords: carbon cycle, soil respiration, stable isotope, atmosphere-terrestrial biosphere exchange, chamber measurement

## INTRODUCTION

The  $^{13}\text{C}/^{12}\text{C}$  ratio (commonly expressed in simplified form,  $\delta^{13}\text{C}$ ) of atmospheric CO<sub>2</sub> provides important information about the carbon budget in global scale (e.g., Tans *et al.*, 1993) and also in ecosystem scale (e.g., Yakir and Sternberg, 2000). Those methods are based on the mass balance of total CO<sub>2</sub> and  $^{13}\text{CO}_2$  in the atmosphere. Atmospheric CO<sub>2</sub> mole fraction ( $[\text{CO}_2]$ ) and the  $\delta^{13}\text{C}$  of CO<sub>2</sub> both fluctuate within the terrestrial biosphere in response to photosynthetic and respiratory fluxes.

Soil respiration is the largest flux component from the terrestrial ecosystem to the atmosphere. Despite its importance, information about temporal and spatial variations in  $\delta^{13}\text{C}$  of soil respiration ( $\delta^{13}\text{C}_{\text{R-soil}}$ ) and its controlling factors under natural condition is lacking because of technical difficulties. Our ultimate goal was to clarify the spatial and temporal distribution of  $\delta^{13}\text{C}_{\text{R-soil}}$  in the widespread terrestrial ecosystem. This would ensure functioning of  $^{13}\text{C}$  signature as an independent variable to be used in advanced study of atmosphere-terrestrial bio-

sphere CO<sub>2</sub> exchange. At present circumstances, our primary motivation was to develop methodology and effective measurement strategy for observation of temporal and spatial variations in  $\delta^{13}\text{C}_{\text{R-soil}}$ .

The main issues in the determination of the  $\delta^{13}\text{C}_{\text{R-soil}}$  are how to eliminate measurement artifacts (Högberg *et al.*, 2005). In the present paper, we have defined “soil-respired CO<sub>2</sub>” as that which diffuses across the soil-atmosphere interface and “soil CO<sub>2</sub>” as the CO<sub>2</sub> found within the soil. The  $\delta^{13}\text{C}_{\text{R-soil}}$  is controlled not only by CO<sub>2</sub> produced within the soil but also by diffusion. Under steady-state conditions,  $\delta^{13}\text{C}_{\text{R-soil}}$  equals the integrated value of the  $\delta^{13}\text{C}$  produced within the soil, but the  $\delta^{13}\text{C}$  of soil CO<sub>2</sub> would be enriched by around 4.4‰ from the  $\delta^{13}\text{C}_{\text{R-soil}}$  because of isotopic fractionation during diffusion (Amundsen *et al.*, 1998). Chamber-based measurements can lead to physical disturbance of the CO<sub>2</sub> gradient at the soil-atmosphere interface and may thus produce artifacts in the estimation of soil CO<sub>2</sub> efflux (Davidson *et al.*, 2002) and thus, possibly in the estimation of  $\delta^{13}\text{C}_{\text{R-soil}}$ . In particular, any pressure anomaly would cause mass flow of soil CO<sub>2</sub> with an anomalously enriched  $\delta^{13}\text{C}$  value. This effect would lead to artifact in the  $\delta^{13}\text{C}_{\text{R-soil}}$  determination. In addition, alteration of  $[\text{CO}_2]$  in the chamber would change the  $[\text{CO}_2]$  gradient

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at the soil surface. It would potentially affect the ratio of  $^{13}\text{CO}_2$  to  $^{12}\text{CO}_2$  that diffuse across the soil-atmosphere interface, but there is no theoretical analysis available for the effect presently. It should be noted that precise quantification of the magnitude of the bias in the  $\delta^{13}\text{C}_{\text{R-soil}}$  resulted from the measurement artifacts was extremely difficult because of difficulties in accumulating sufficient information at specific times and locations (e.g., vertical distributions of the diffusion coefficient, *in situ* decomposition rates, and the isotopic composition of the decomposed material). Hence we must carefully plan the field-experiment.

The potential problems in the previous sampling techniques had been pointed out in recent studies (e.g., Högberg *et al.*, 2005; Mortazavi *et al.*, 2004; Ohlsson *et al.*, 2005). For example, Flanagan *et al.* (1996) and Buchmann *et al.* (1997) collected air in closed chambers into glass flasks during the recovering stage after scrubbing  $\text{CO}_2$  by soda lime. This technique would unavoidably involve significant disturbance in  $\text{CO}_2$  gradient at soil-atmosphere interface, consequently generated concern for giving influence in isotopic fractionation during diffusion. Ohlsson *et al.* (2005) tested two different sampling approaches with and without initial flushing of static closed-chamber interior with synthetic air that contained very low  $\text{CO}_2$ . They reported that lowering of the initial  $\text{CO}_2$  fraction induced significant enrichment ( $>4\%$ ) in the  $\delta^{13}\text{C}_{\text{R-soil}}$ . As to the cause of the  $\delta^{13}\text{C}_{\text{R-soil}}$  enrichment, the explanation was suggested that the initial lowering of the  $[\text{CO}_2]$  caused a disturbance to the  $\text{CO}_2$  diffusion process within the soil-chamber system. The experimental configuration of  $[\text{CO}_2]$  gradient for the case of initial- $\text{CO}_2$ -lowering was similar to those of Flanagan *et al.* (1996), and Buchmann *et al.* (1997). Ohlsson *et al.* (2005) also reported that the estimate of  $\delta^{13}\text{C}_{\text{R-soil}}$  was not affected by the increase of  $[\text{CO}_2]$  up to more than  $2000 \mu\text{mol}\cdot\text{mol}^{-1}$  for the non-flushed chamber though the apparent  $\text{CO}_2$  efflux in the chamber was significantly declined. The result suggested that the acceptable  $[\text{CO}_2]$  range for the  $\delta^{13}\text{C}_{\text{R-soil}}$  determination was larger than that for the determination of soil  $\text{CO}_2$  efflux.

Mortazavi *et al.* (2004) proposed new field-based sampling approach using open-top-mini-towers. This approach was developed mainly for the precise estimation of  $\delta^{18}\text{O}$ , oxygen stable isotope ratio, of soil respired  $\text{CO}_2$ . But, as for the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation, practical advantage of the approach over the usual closed-chamber sampling was not discussed.

Bowling *et al.* (2003) and McDowell *et al.* (2004) employed static closed chambers coupled with a flask sampling system for the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation with consideration for measurement artifacts related to pressure anomaly and to  $[\text{CO}_2]$  enrichment in the chamber headspace. But they did not conduct experimental vali-

ation of the influence of the  $[\text{CO}_2]$  enrichment.

$\delta^{13}\text{C}_{\text{R-soil}}$  has been often estimated from vertical profiles of  $[\text{CO}_2]$  and  $\delta^{13}\text{C}$  of soil  $\text{CO}_2$  (e.g., Mortazavi *et al.*, 2004; Pendall *et al.*, 2005). This approach assumed that the respiratory end-member  $\delta^{13}\text{C}$  value was constant with depth. The profiles of soil  $\text{CO}_2$  and its  $\delta^{13}\text{C}$  were generated from samples collected below the surface soil layer. Hence the estimates by this approach would hardly include the influence of surface litter decomposition (Mortazavi *et al.*, 2004). In seasonal vegetation types like deciduous forest or in tropical ecosystem, decomposition of surface litter contributes largely to the total soil  $\text{CO}_2$  efflux. Therefore application of this method requires consideration according to the aim of study.

Against this background, we developed an air sampling system optimized for the collection of soil-respired  $\text{CO}_2$  and tested the potential influence from the  $[\text{CO}_2]$  enrichment in the chamber headspace on the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation by using measurements of  $[\text{CO}_2]$  and  $\delta^{13}\text{C}$  under the natural environment with higher precision than the previous studies.

## METHODS

### Field sampling

*Site description* We performed our measurements at the Tomakomai Flux Research site ( $42^\circ44' \text{ N}$ ,  $141^\circ31' \text{ E}$ ), Japan. The predominant tree species was 45-year-old Japanese larch. The soil was homogeneous and was classified as an immature Volcanogenous Regosol.

*Automated soil chamber system* We employed an already-existing multi-channel automated chamber system for soil  $\text{CO}_2$  efflux measurement combined with a newly-developed flask sampling system optimized for collecting soil-respired  $\text{CO}_2$ . The automated chamber system was designed to minimize the influence of physical disturbance in soil-atmosphere interface. The details of the chamber system are described by Liang *et al.* (2003) and Liang *et al.* (2004). The dimensions of each chamber were  $0.9 \times 0.9 \text{ m}$ , with a height of  $0.5 \text{ m}$ . The large volume, small vent, and slow movement of the pneumatically actuated lids effectively minimized pressure anomalies inside the chambers during their operation. The flask sampling system described in the following section was located on a wooden slatted drainboard more than  $2 \text{ m}$  apart from the chambers. The multi-channel automated chamber system was used to monitor  $[\text{CO}_2]$  in the chamber during the flask sampling.

*Flask sampling system* A schematic diagram of the sampling system is shown in Fig. 1. The soil chamber was placed in series in a closed loop with a  $\text{Mg}(\text{ClO}_4)_2$  water trap, a diaphragm pump (Model-MOA, GAST Mfg., Inc., Benton Harbor, MI, USA), an assembly of four glass flasks ( $750\text{-mL}$ , each with two vacuum stopcocks with a

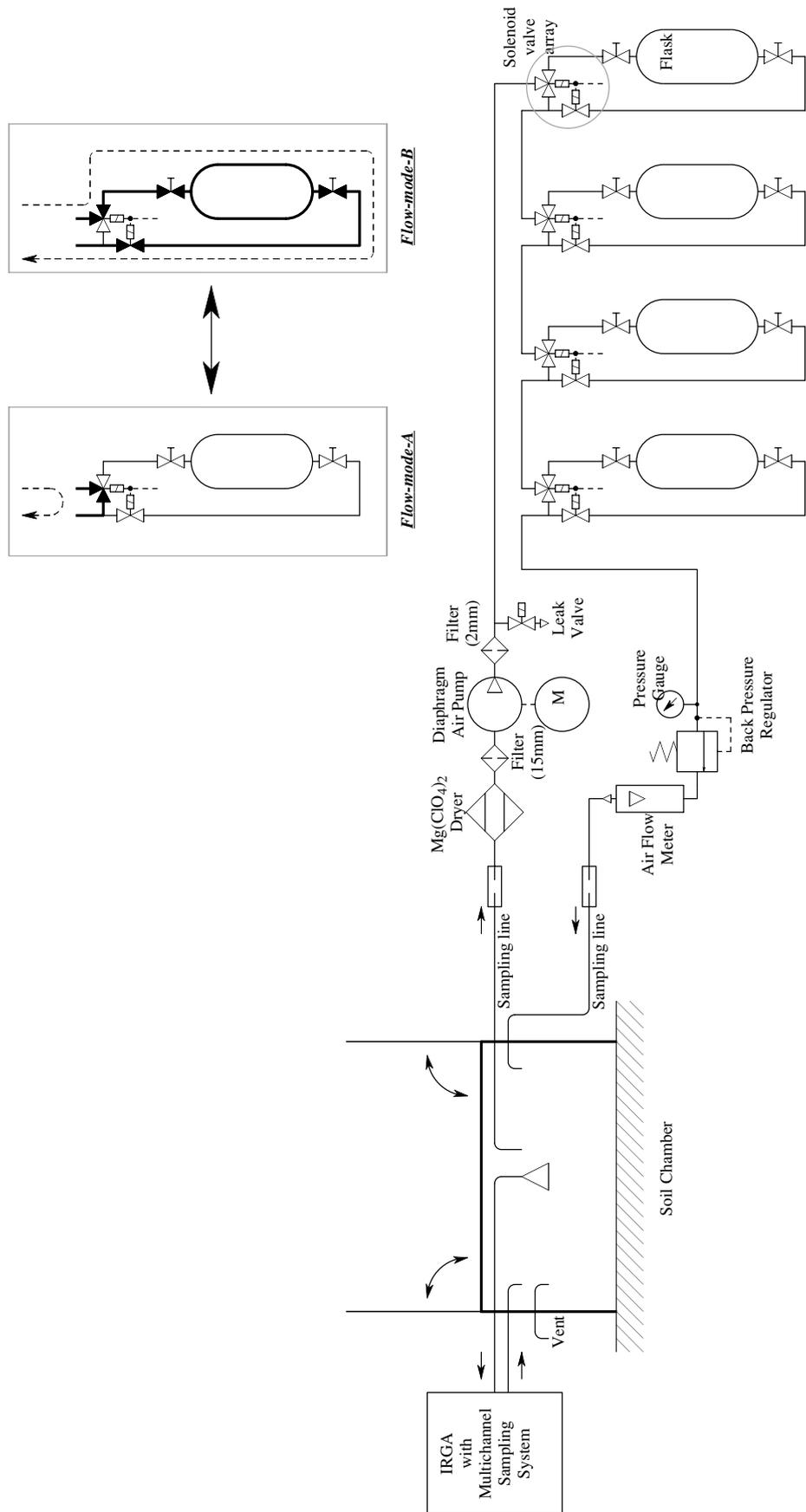


Fig. 1. Schematic diagram of flask sampling system used in measurements of  $\delta^{13}\text{C}_{\text{R-soil}}$ .

Viton® O-ring seal at both ends; Koshin Rikagaku Seisakusho, Tokyo, Japan), a back-pressure regulator (Model-6800AL, KOFLOC, Tokyo, Japan), and a flowmeter (Model-RK1000, KOFLOC, Tokyo, Japan). All the glass flasks were connected in series. Solenoid valve arrays (USB3-6-2 and USG3-6-2, CKD, Tokyo, Japan) were placed in parallel with each flask to switch the flow path instantaneously between two modes without stopping the airstream. The airstream bypassed the flasks in flow mode A and passed through the flasks in flow mode B (Fig. 1). Inflow to and outflow from the soil chamber were balanced throughout a sampling operation. The sampling system and the chambers were connected with polyethylene/aluminum composite tubes (DK-1300, 6-mm O.D., 10-m in length, Nitta-Moore, Tokyo, Japan). We designed the flask sampling system to collect sample air into four flasks under continuously pressurized condition without introducing any pressure fluctuation in the chamber headspace. Sample collection under positive pressure made it possible to introduce samples into analyzers directly without contact pumping system that likely involves changing quality of the samples. The sampling system was covered with plastic box (dimension of basal plane was 0.75 m × 0.5 m) and was located on a wooden slatted drainboard more than 2 m apart from the nearest chamber. Hence the disturbance in soil CO<sub>2</sub> efflux due to the covering of soil surface was unlikely. Great care was taken to avoid contaminating the atmosphere around the chambers with the CO<sub>2</sub> contained in human breath.

We collected air samples using the following procedure. About 5 min before closing the chamber lid, all flask stopcocks were opened, and all solenoid valves were set to flow mode B. We then ran the pump to flush all the flasks and tubing with ambient air from the chamber; 5 sec after closing the lid of the chamber, an upstream solenoid valve array switched to flow mode A to isolate the flasks from the airstream, then stopcocks on both sides of the flask were closed. The other three flasks were isolated from the airstream and closed sequentially from upstream to downstream in the same manner at nearly constant time intervals. The air pressure inside the sampling system was kept constant (at approximately 100 kPa above ambient) by means of a back-pressure regulator. The flow rate of the sample air was about 6 L·min<sup>-1</sup> during the sampling. Overall collection times were shorter than 810 sec.

#### Laboratory analysis

We analyzed the [CO<sub>2</sub>] and the δ<sup>13</sup>C of the CO<sub>2</sub> in the air samples in a laboratory of the National Institute for Environmental Studies. The [CO<sub>2</sub>] values in the samples were determined using a nondispersive infrared gas analyzer (LI-6252, LI-COR, Lincoln, NE, USA). The sam-

ple air was introduced into a cell of the analyzer through a cryogenic drying trap (glass U-tube immersed in dry ice-ethanol bath) and a mass flow controller (Model SEC-4400, Horiba STEC, Kyoto, Japan) by pressure difference between the sample flask and the ambient air. We estimated the precision of the [CO<sub>2</sub>] analysis to be better than 0.10 μmol·mol<sup>-1</sup>. After analyses of [CO<sub>2</sub>], we performed cryogenic extraction of CO<sub>2</sub> using a glass vacuum line for our isotopic measurement. The principle of this extraction is similar to that described by Vaughn *et al.* (2004). The extracted CO<sub>2</sub> was introduced into an isotope-ratio mass spectrometer (Delta-PLUS, Thermo Electron Co., Waltham, MA, USA) using variable-volume, dual-inlet devices. We corrected for the presence of N<sub>2</sub>O in the sample CO<sub>2</sub> using measured [N<sub>2</sub>O]/[CO<sub>2</sub>] values for each sample and a correction factor that accounted for differences in ionization efficiency between CO<sub>2</sub> and N<sub>2</sub>O. The correction factor was individually determined using the approach described by Friedli and Siegenthaler (1988) for each filament in the ion-source of the IRMS after each filament exchange. When N<sub>2</sub>O-correction was not applied, apparent δ<sup>13</sup>C value became more depleted by about 0.2‰ than N<sub>2</sub>O-corrected-value under the ambient levels of [CO<sub>2</sub>] and [N<sub>2</sub>O] (380 μmol·mol<sup>-1</sup> and 317 nmol·mol<sup>-1</sup>, respectively) and magnitude of the N<sub>2</sub>O-effect varies according to [CO<sub>2</sub>]/[N<sub>2</sub>O] ratio in sample air. When the soil N<sub>2</sub>O efflux was negligible, good approximation of [CO<sub>2</sub>]/[N<sub>2</sub>O] would be available by assuming that [N<sub>2</sub>O] was constant at ambient level. If samples were collected in the environment that soil N<sub>2</sub>O efflux was significantly large, for example in cropland or tropical ecosystem, the separation of N<sub>2</sub>O or the [N<sub>2</sub>O] measurement should be performed for precise estimation of δ<sup>13</sup>C<sub>R-soil</sub>.

The <sup>13</sup>C/<sup>12</sup>C ratio (delta notation) was defined as follows:

$$\delta^{13}\text{C}(\text{‰}) = \left\{ \frac{\left( \frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Sample}}}{\left( \frac{^{13}\text{C}}{^{12}\text{C}} \right)_{\text{Standard}}} - 1 \right\} \times 1000. \quad (1)$$

We reported the δ<sup>13</sup>C value using the Vienna-PDB scale. The overall precision of the δ<sup>13</sup>C analysis (including the extraction process) was estimated to be better than 0.02‰.

#### Determination of δ<sup>13</sup>C of soil-respired CO<sub>2</sub>

To determine the δ<sup>13</sup>C<sub>R-soil</sub> value, we used a simple mixing model usually called the “Keeling plot” (Keeling, 1958). The usability and the limitations of the Keeling plot approach in the ecological studies are described by Pataki *et al.* (2003). In this study, we assumed that the relationship between [CO<sub>2</sub>] and δ<sup>13</sup>C in the chambers was expressed in the “Keeling plot” as follows:

Table 1. Distribution of coefficient of determination ( $R^2$ ) for different  $[CO_2]$  increments. The number of sample ( $n$ ) was 62. The smallest value of  $r^2$  was 0.995.

	$\Delta[CO_2] < 50$ ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	$50 < \Delta[CO_2] < 100$ ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	$100 < \Delta[CO_2] < 150$ ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	$150 < \Delta[CO_2] < 200$ ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )	$200 < \Delta[CO_2]$ ( $\mu\text{mol}\cdot\text{mol}^{-1}$ )
$R^2 > 0.9999$	1	7	11	11	5
$0.9999 > R^2 > 0.999$	7	6	7	1	1
$0.999 > R^2$	4	1	(none)	(none)	(none)
Total	12	14	18	12	6

$\Delta[CO_2]$  was defined as a difference in the  $[CO_2]$  between start and end of each sampling.

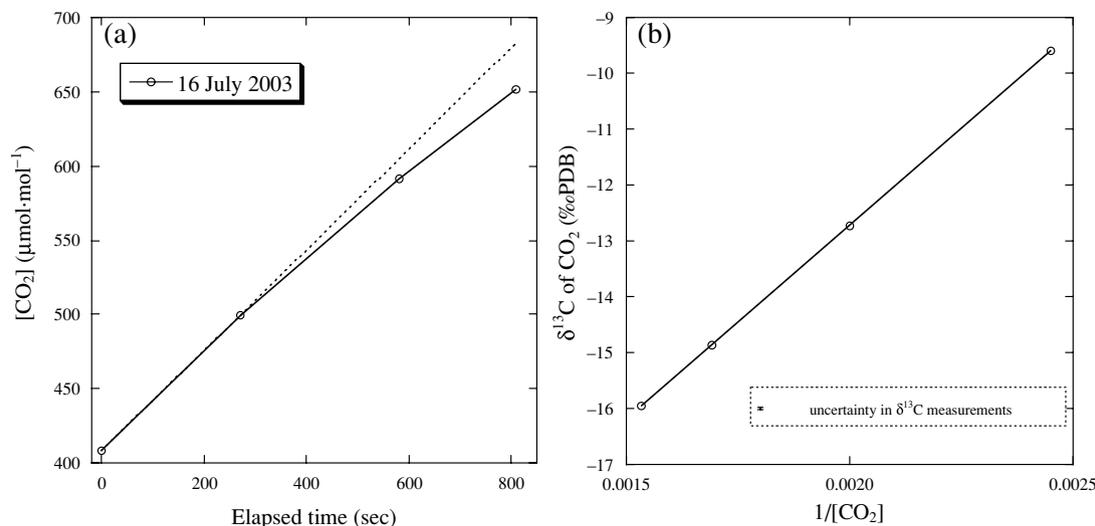


Fig. 2. (a) Relationship between  $[CO_2]$  in the chamber and time elapsed from start of the sampling for the case with the large  $[CO_2]$  increment. Hashed line represent a linear extrapolation calculated from the first and the second data point. (b) Relationship between  $\delta^{13}C$  and  $1/[CO_2]$  for the same samples shown in (a). Error bar represent the analytical uncertainty in our  $\delta^{13}C$  measurement. All the data points locate completely on the regression line.

$$\delta^{13}C_{Ch} = \frac{[CO_2]_{BG}}{[CO_2]_{Ch}} (\delta^{13}C_{BG} - \delta^{13}C_{R-soil}) + \delta^{13}C_{R-soil} \quad (2)$$

where the subscripts  $Ch$  and  $BG$  represent the atmosphere in the chambers and the background atmosphere, respectively. Assuming that there are no changes in both  $\delta^{13}C_{BG}$  and  $\delta^{13}C_{R-soil}$  between the start and end of each sampling period, the y-intercept of the linear regression of  $\delta^{13}C$  versus  $1/[CO_2]$  observed in the chamber represents the  $\delta^{13}C_{R-soil}$ . We used geometric mean (model II) regressions for this analysis (Sokal and Rohlf, 1995). The error in the determination of the y-intercept in each individual Keeling plot was estimated using a weighted Deming regression (Deming, 1943) (see Appendix).

## RESULTS AND DISCUSSION

Though our system could avoid pressure anomaly in

the chamber effectively, the potential influence of the  $[CO_2]$  enrichment was unavoidable in the sampling. While collecting samples with wider  $[CO_2]$  range contributes to minimize standard error of  $\delta^{13}C_{R-soil}$ , it raises the risk of potential influence related to the  $[CO_2]$  enrichment. If the  $[CO_2]$  enrichment has any influence on  $\delta^{13}C_{R-soil}$ , the influence would become greater in higher  $[CO_2]$ . It should reflect in the linearity of  $\delta^{13}C$ -vs- $1/[CO_2]$  plot. For this reason, we tested the linearity of the plot for the influence of the  $[CO_2]$  enrichment.

We calculated the correlation coefficient for 62 Keeling plots that consist of four data points on individual sampling. We categorized all the cases into 5 groups by the  $[CO_2]$  increment ( $\Delta[CO_2]$ ) and sorted the number of cases ( $n$ ) into corresponding range of coefficient of determination ( $R^2$ ) (Table 1). The values of  $R^2$  were greater than 0.995 for all the 62 cases. As for the cases with  $[CO_2]$  range greater than  $100 \mu\text{mol}\cdot\text{mol}^{-1}$ , the value of  $R^2$  was never to be less than 0.999. We found tendency that the

value of  $R^2$  become slightly lower in the lesser  $\Delta[\text{CO}_2]$  range. This feature was most likely due to the analytical uncertainties became greater relative to the variation amplitudes.

We then tested the influence of an excessive  $\Delta[\text{CO}_2]$  in the Keeling plot. We here afford an instance of actual observational results. Figure 2(a) shows the relationship between  $[\text{CO}_2]$  in the chamber and time elapsed from the start of the sampling for the case of the greatest  $\Delta[\text{CO}_2]$ . We found a significant decrease in the  $[\text{CO}_2]$  increasing rate per unit time as the  $[\text{CO}_2]$  became higher. This tendency was found for the full range of our observed results. Figure 2(b) shows the relationship between  $\delta^{13}\text{C}$  and  $1/[\text{CO}_2]$  for the same samples shown in Fig. 2(a). In all our measurements, the relationship was linear, with no systematic dependency on the value of  $1/[\text{CO}_2]$ . This suggested that the estimates of  $\delta^{13}\text{C}_{\text{R-soil}}$  was insusceptible to the influence of the  $[\text{CO}_2]$  enrichment under our measurement conditions. Our results were consistent with the finding of Ohlsson *et al.* (2005) that the linear relationship between  $1/[\text{CO}_2]$  and  $\delta^{13}\text{C}$  was preserved as chamber  $[\text{CO}_2]$  rose up to substantially high level starting from ambient condition. Those results suggested that acceptable range of  $\Delta[\text{CO}_2]$  for the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation was considerably larger than that for soil  $\text{CO}_2$  efflux measurements, though it was just an empirical fact and had not been validated theoretically at this point in time.

Our results have implications for the adequate experimental configuration of chamber-based sampling for the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation. The standard error of the  $\delta^{13}\text{C}_{\text{R-soil}}$  determined by the Keeling plot approach was constrained by uncertainties in the measured values of  $\delta^{13}\text{C}$  and  $[\text{CO}_2]$ , on the number of samples ( $n$ ), and on the  $\Delta[\text{CO}_2]$ . In general, reduction of the uncertainties in  $\delta^{13}\text{C}$  measurements was most effective means for decreasing standard error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation (see Appendix). In practical terms, improvement of analytical precision could contribute to shorten the time of sampling operation and to minimize potential influence derived by chamber effects (influence of artificial change in environmental factors in the chambers; e.g., change in  $\text{CO}_2$  diffusivity due to temperature-rise, solution of  $\text{CO}_2$  into condensation at chamber-wall etc.). However, the uncertainty in measured  $\delta^{13}\text{C}$  depends primarily on analytical facilities of experimenters, and thus the fundamental improvement of analytical precision would be cost-intensive in many cases. In the generality of cases, the extension of the  $\Delta[\text{CO}_2]$  is a cost-effective means for reduction of the  $\delta^{13}\text{C}_{\text{R-soil}}$  uncertainty as far as linearity between  $1/[\text{CO}_2]$  and  $\delta^{13}\text{C}$  is preserved.

The uncertainty in the  $\delta^{13}\text{C}_{\text{R-soil}}$  is affected also by difference in  $[\text{CO}_2]$  interval of data. When the data distribution is biased toward the higher  $[\text{CO}_2]$ , error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimates is to be smaller than that for the case

of even distribution (see Appendix). However we considered that the improvement of the  $\delta^{13}\text{C}_{\text{R-soil}}$  error by the extremely biased sampling interval is unfavorable in practical field experiment because this involves having difficulty in testing the linearity between  $1/[\text{CO}_2]$  and  $\delta^{13}\text{C}$  in the entire sampling range. Since Keeling plot approach requires assumption of two end-member simple mixing, we regard the linearity test as an essential prerequisite to ascertain the validity of its results in general terms.

The reduction of the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation by increasing number of samples ( $n$ ) is a labor-intensive approach involving considerable expense of throughput of the  $\delta^{13}\text{C}_{\text{R-soil}}$  determination (see Appendix). In ecosystem with highly heterogeneous environment, for example in natural forest, estimating of representing value for ecosystem-scale could be a higher priority than the reduction of the error in chamber-scale. The lowered throughput can be a major obstacle to obtain the mean  $\delta^{13}\text{C}_{\text{R-soil}}$  estimates with better spatial-representativeness in natural heterogeneity by increasing number of sampling locations. Increasing of  $n$  for the each single  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation also involves rising of total sampling cost in the experiment. Hence, we do not recommend this strategy except for the case that study subject exists in chamber-scale processes.

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#### APPENDIX: QUANTITATIVE EVALUATION OF UNCERTAINTY IN $\delta^{13}\text{C}$ OF RESPIRATORY COMPONENT DETERMINED BY KEELING PLOT APPROACH

In this appendix, we devote extended discussion to technical aspects of experimental configuration for the Keeling plot approach. The standard error of the  $\delta^{13}\text{C}_{\text{R-soil}}$  determined by the Keeling plot approach depends on uncertainties in the measured values of  $\delta^{13}\text{C}$  and [CO<sub>2</sub>], on the number of samples ( $n$ ), and on the  $\Delta[\text{CO}_2]$ . In this study, the error in the determination of the  $y$ -intercept in each individual Keeling plot was estimated using a weighted Deming regression (Deming, 1943). The main concept of this regression method is orthogonal least-squares estimates to minimize the squared deviation of the observed data from the regression line. The reason why we choose this regression method is that this method was useful to estimate standard error in estimated  $y$ -intercept ( $\delta^{13}\text{C}_{\text{R-soil}}$ ) reflecting measurement errors of both  $x$ -variable ( $1/[\text{CO}_2]$ ) and  $y$ -variable ( $\delta^{13}\text{C}$ ). Computation of the method can be performed by function of statistical software packages (e.g., SAS, Method Validator, Cbstat etc.). Standard errors for the estimates are obtained by so-called Jackknife method in this regression method. Comparison of the Deming regression method and other regression methods was made by Cornbleet and Gochman (1979) and Linnet (1993).

Now we aimed to reduce the error of  $\delta^{13}\text{C}_{\text{R-soil}}$  to 0.1‰ tentatively. To determine the appropriate experimental configuration for precise determination of  $\delta^{13}\text{C}_{\text{R-soil}}$ , we tested the standard error of the  $\delta^{13}\text{C}_{\text{R-soil}}$  with simulated parameters. We used the assumption of simple mixing, with constant start and end values of  $\delta^{13}\text{C}_{\text{BG}}$  and  $\delta^{13}\text{C}_{\text{R-soil}}$ , to create hypothetical sample data. We then set [CO<sub>2</sub>]<sub>BG</sub>,  $\delta^{13}\text{C}_{\text{BG}}$  and  $\delta^{13}\text{C}_{\text{R-soil}}$  to 370  $\mu\text{mol}\cdot\text{mol}^{-1}$ , -8 and -27‰ PDB, respectively. We also assumed that the interval of [CO<sub>2</sub>] between adjacent data points was constant and that the uncertainty in [CO<sub>2</sub>] was 0.10  $\mu\text{mol}\cdot\text{mol}^{-1}$ .

Figure A1(a) shows the relationship between the standard error in  $\delta^{13}\text{C}_{\text{R-soil}}$  and the  $\Delta[\text{CO}_2]$  for five fixed values (0.02, 0.05, 0.10, 0.20, and 0.50‰) of uncertainty in the measured  $\delta^{13}\text{C}$  ( $u_\delta$ ). When  $n = 4$ , the  $\Delta[\text{CO}_2]$  required for us to obtain an error of 0.10‰ were 120  $\mu\text{mol}\cdot\text{mol}^{-1}$  for  $u_\delta = 0.02\%$ , 361  $\mu\text{mol}\cdot\text{mol}^{-1}$  for  $u_\delta = 0.05\%$ , and 1173

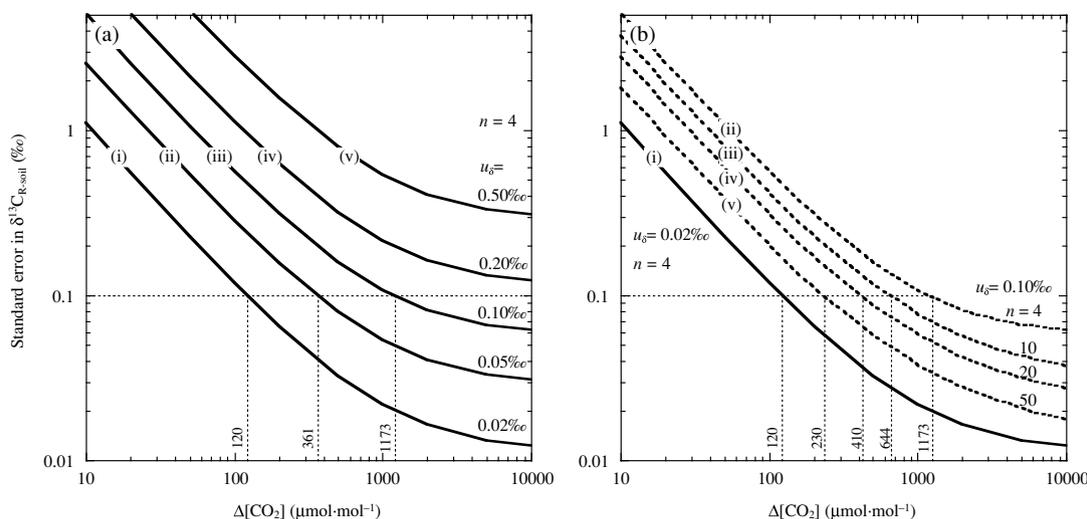


Fig. A1. (a) Relationship between the standard error in  $\delta^{13}\text{C}_{\text{R-soil}}$  and the range of  $[\text{CO}_2]$  increment ( $\Delta[\text{CO}_2]$ ) for five fixed values ((i) 0.02, (ii) 0.05, (iii) 0.10, (iv) 0.20, and (v) 0.50‰) of uncertainty in the measured  $\delta^{13}\text{C}$  ( $u_\delta$ ) when  $n = 4$ . (b) Effect of  $n$ -increase in reducing standard error of Keeling plot intercept. (i)  $n = 4$  with  $u_\delta = 0.02\%$ , (ii)  $n = 4$  with  $u_\delta = 0.10\%$ , (iii)  $n = 10$  with  $u_\delta = 0.10\%$ , (iv)  $n = 20$  with  $u_\delta = 0.10\%$ , and (v)  $n = 50$  with  $u_\delta = 0.10\%$ , respectively.

$\mu\text{mol}\cdot\text{mol}^{-1}$  for  $u_\delta = 0.10\%$ , respectively. We could not obtain a 0.1‰ error in  $\delta^{13}\text{C}_{\text{R-soil}}$  with  $u_\delta$  greater than 0.20‰ in the case of  $n = 4$ . This result shows reducing of the analytical error in  $\delta^{13}\text{C}$  is essential to reduce the required  $\Delta[\text{CO}_2]$  for the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation.

Here we briefly note the effect of reducing uncertainty in  $[\text{CO}_2]$  ( $u_C$ ) on the  $\delta^{13}\text{C}_{\text{R-soil}}$  estimation. Assuming that four samples with  $[\text{CO}_2] = 370, 470, 570$  and  $670 \mu\text{mol}\cdot\text{mol}^{-1}$  were collected from chamber, the standard error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  was to be 0.05‰ when  $u_C = 0.1 \mu\text{mol}\cdot\text{mol}^{-1}$  and  $u_\delta = 0.02\%$ . If the  $u_\delta$  was increased by ten times, i.e.,  $u_\delta = 0.2\%$ , and  $u_C$  was constant ( $=0.1 \mu\text{mol}\cdot\text{mol}^{-1}$ ), the standard error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  was to be 0.46‰. On the other hand, if the  $u_C$  was increased by ten times, i.e.,  $u_C = 1 \mu\text{mol}\cdot\text{mol}^{-1}$ , and  $u_\delta$  was constant ( $=0.02\%$ ), the standard error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  was to be 0.17‰. Those results suggests that the  $u_\delta$  constrains the standard error in the  $\delta^{13}\text{C}_{\text{R-soil}}$  more strongly than the  $u_C$ .

Then we tested the influence of difference in interval of data. We assumed three cases with different  $[\text{CO}_2]$  in-

tervals within the same total  $\Delta[\text{CO}_2]$ ;  $[\text{CO}_2]$  of four samples were (i) 370, 470, 570 and  $670 \mu\text{mol}\cdot\text{mol}^{-1}$ , (ii) 370, 420, 470 and  $670 \mu\text{mol}\cdot\text{mol}^{-1}$  and (iii) 370, 570, 620 and  $670 \mu\text{mol}\cdot\text{mol}^{-1}$ , respectively. When  $u_C = 0.1 \mu\text{mol}\cdot\text{mol}^{-1}$  and  $u_\delta = 0.02\%$ , standard errors in the  $\delta^{13}\text{C}_{\text{R-soil}}$  for case (i), (ii) and (iii) were 0.047, 0.051 and 0.043‰, respectively. This result demonstrates that data point in higher  $[\text{CO}_2]$  range have more power in determining magnitude of the  $\delta^{13}\text{C}_{\text{R-soil}}$  error.

Assuming that the available  $\Delta[\text{CO}_2]$  was constant, the required sample size to obtain the same level of the  $\delta^{13}\text{C}_{\text{R-soil}}$  error should be approximately proportional to the square of the magnitude of the  $u_\delta$ . For example, using four samples with a  $u_\delta$  value of 0.02‰ was almost equivalent to that of 100 samples with a  $u_\delta$  value of 0.10‰ in obtaining an arbitrary level of the  $\delta^{13}\text{C}_{\text{R-soil}}$  precision. The example in Fig. A1(b) illustrates the effect of increasing number of samples ( $n$ ) in the improvement of the  $\delta^{13}\text{C}_{\text{R-soil}}$  uncertainty.