JOURNAL OF GEOPHYSICAL RESEARCH, VOL. 113, G01022, doi:10.1029/2007JG000478, 2008

Variability in carbon stable isotope ratio of heterotrophic respiration in a deciduous needle-leaf forest

Yoshiyuki Takahashi,[1] Naishen Liang,[1] Ryuichi Hirata,[1] Toshinobu Machida,[1] and Yasumi Fujinuma[1]

Received 1 May 2007; revised 10 September 2007; accepted 1 November 2007; published 13 March 2008.

[1] We investigated spatial and temporal variability in the carbon stable isotope ratio ($\delta^{13}C$) of soil heterotrophic respiration in a deciduous needle-leaf forest in Japan for 3 years. We used high-precision isotope measurement coupled with a sampling system optimized for soil respiration to capture this variability under natural conditions. The limitations of chamber-based measurements combined with spatial variation created a representation error that prevented precise estimates of flux-weighted mean $\delta^{13}C$, but we could nonetheless characterize the $\delta^{13}C$ variations intrinsic to heterotrophic respiration. In the absence of root respiration, $\delta^{13}C$ exhibited significant seasonal variation, with a greater range than in previous models. In a root-exclusion plot, $\delta^{13}C$ was lowest at high temperatures but showed a different seasonal course from that of CO_2 efflux. A simple model explained the seasonal variation in $\delta^{13}C$ using interpool differences in $\delta^{13}C$ of decomposed organic matter, in the temperature dependence of decomposition rates, and in the seasonal changes in pool size. The characteristic seasonality of $\delta^{13}C$ appears to be associated with the properties of the forest, including litterfall patterns.

Citation: Takahashi, Y., N. Liang, R. Hirata, T. Machida, and Y. Fujinuma (2008), Variability in carbon stable isotope ratio of heterotrophic respiration in a deciduous needle-leaf forest, *J. Geophys. Res.*, *113*, G01022, doi:10.1029/2007JG000478.

1. Introduction

[2] The carbon stable isotope ratio (commonly expressed in simplified form, $\delta^{13}C$, whose definition is described in section 2.4 of this paper) of atmospheric CO_2 provides important information about the global carbon budget [e.g., *Tans et al.*, 1993]. Combined with consecutive measurements of the atmospheric CO_2 mixing ratio ($[CO_2]$), time series of the $\delta^{13}C$ values of atmospheric CO_2 have been used to estimate the relative roles of the oceans and terrestrial biosphere as net CO_2 sinks [*Heimann and Keeling*, 1989; *Ciais et al.*, 1995a, 1995b; *Francey et al.*, 1995; *Keeling*, 1995; *Fung et al.*, 1997; *Bousquet*, 1999a, 1999b; *Rayner et al.*, 1999]. This method is based on the mass balance of CO_2 and $^{13}CO_2$ in the atmosphere. Atmospheric $[CO_2]$ and the $\delta^{13}C$ of CO_2 both fluctuate within the terrestrial biosphere in response to photosynthetic and respiratory fluxes. If the exchange of CO_2 and $^{13}CO_2$ between the atmosphere and the terrestrial biosphere operates under steady-state conditions, the flux-weighted mean $\delta^{13}C$ of the CO_2 assimilated by photosynthesis equals the flux-weighted mean $\delta^{13}C$ of the CO_2 released by respiration on an annual mean basis. However, the $\delta^{13}C$ of atmospheric CO_2 has been changed due to the release of ^{13}C-depleted CO_2 by combustion of fossil fuels. Hence, the time difference between photosynthetic uptake and respiratory release of CO_2 by the terrestrial biosphere causes "isotopic disequilibrium" [e.g., *Fung et al.*, 1997]. Estimates of carbon budgets using the $\delta^{13}C$ of atmospheric CO_2 depend strongly on the choice of values for this disequilibrium [*Ciais et al.*, 1999]. When we use atmospheric tracer transport models instead of a single-box model of the atmosphere, the spatial and temporal distribution of the isotope disequilibrium must also be estimated.

[3] Evaluating the isotope disequilibrium in the atmosphere–biosphere CO_2 exchange requires an accurate understanding of the isotopic signature of the respiratory CO_2 flux from the terrestrial ecosystem into the atmosphere (F_{ba} of equation (1a) in *Fung et al.* [1997]). If we define the counter-flux of F_{ba} (F_{ap}) in this equation as net carbon assimilation, then F_{ba} equals ecosystem respiration. This ecosystem respiration is subdivided into two components: autotrophic respiration by plants and heterotrophic respiration resulting from microbial decomposition of dead organic matter. The autotrophic respiration is directly linked to new carbon assimilated by means of photosynthesis, hence the isotopic signature from autotrophic respiration would immediately reflect the change in photosynthetic isotope discrimination. Assuming that an isotopic equilibrium exists between autotrophic respiration and carbon fixed by photosynthesis, F_{ap} can be identified to net primary productivity (NPP) and F_{ba} can be identified to heterotrophic respiration [*Ciais et al.*, 2005]. To investigate the seasonal and interannual variability of the isotope disequilibrium related to atmosphere–biosphere CO_2 exchange and its spatial distribution, it is important to accurately assess the $\delta^{13}C$ of heterotrophic respiration. It must be noted that the

[1]Center for Global Environmental Research, National Institute for Environmental Studies, Tsukuba, Japan.

0148-0227/08/2007JG000478$09.00

modeled values of isotopic disequilibrium between newly formed phytomass and soil-respired CO_2 are typically smaller than 1‰ (e.g., 0.33 to 0.56‰ [*Heimann and Keeling*, 1989; *Ciais et al.*, 1995a, 1999; *Fung et al.*, 1997; *Scholze et al.*, 2003]).

[4] Recent studies have revealed that $\delta^{13}C$ of ecosystem respiration ($\delta^{13}C_R$) or soil respiration ($\delta^{13}C_{R\text{-}soil}$) has significant spatial and temporal variability. *Ekblad and Högberg* [2001] and *Ekblad et al.* [2005] found that $\delta^{13}C$ of soil respiration in a boreal mixed coniferous forest had seasonal variation in magnitude of nearly 5‰, and suggested potential linkage between the $\delta^{13}C$ and above ground weather conditions 1–4 days before. *Bowling et al.* [2002] reported that $\delta^{13}C$ of ecosystem respiration observed at six coniferous forests showed strong linkage with the vapor saturation deficit of air 5–10 days earlier. The results of investigation made by *McDowell et al.* [2004] showed that the $\delta^{13}C$ of soil respiration had a variability associated with meteorological factors although the $\delta^{13}C$ of ecosystem respiration was not controlled solely by either aboveground and belowground processes. In those studies, the variation in $\delta^{13}C$ of soil respiration and/or ecosystem respiration was interpreted to reflect the fluctuation in photosynthetic isotope discrimination via autotrophic respiration within timescale of several days. In ecological aspects, the change of the $\delta^{13}C$ of autotrophic respiration in rapid response to environmental factors has provided useful information about carbon cycle in terrestrial ecosystem. But we considered that correct understanding of natural variations in the $\delta^{13}C$ of heterotrophic respiration would be desirable in the studies of large-scale atmosphere-terrestrial biosphere CO_2 exchange using ^{13}C information. Despite its importance, there is lacking in reliable measurement of the $\delta^{13}C$ of heterotrophic respiration under natural conditions. Although *Fung et al.* [1997] estimated the seasonal range of variation in the isotopic signature of F_{ba} to be less than 0.3‰ everywhere in the world using their model, this conclusion has not been verified by field observations.

[5] Against this background, we set out to observationally validate model predictions of the seasonality of the $\delta^{13}C$ of heterotrophic respiration [*Fung et al.*, 1997] and of the isotopic disequilibrium [*Fung et al.*, 1997; *Scholze et al.*, 2003]. To support this goal, we must correctly measure the natural variability in $\delta^{13}C_{R\text{-}soil}$. In this study, we conducted the following field observations:

[6] 1. To assess the representativeness of the $\delta^{13}C_{R\text{-}soil}$ values measured by our chamber-based sampling method for a given spatial scale, we examined short-term temporal variations and spatial variations of $\delta^{13}C_{R\text{-}soil}$ with and without the influence of root respiration.

[7] 2. To validate the model prediction that the $\delta^{13}C$ of heterotrophic respiration has insignificant seasonality, we conducted regular fixed-point sampling in a deciduous Japanese needle-leaf forest for nearly 3 years.

2. Materials and Methods

2.1. Site Description

[8] We performed our measurements at the Tomakomai Flux Research site (42°44′N, 141°31′E) on Hokkaido Island, northern Japan, about 15 km inshore from the Pacific Ocean. The site is located in Tomakomai National Forest. The predominant tree species at the site was 45-year-old Japanese larch (*Larix kaempferi* Sarg.), interspersed with Japanese spruce (*Picea jezoensis* Sieb. et Zucc.) and mixed broadleaved species (*Betula ermanii* Cham., *Ulmus davidiana var. japonica*, *Prunus jamasakura* Sied. ex Koidz. etc.). The larch forest dominated 98 ha of the total 117 ha of the forest. In 1999, the overstory density was ca. 1087 stems ha^{-1}, the total basal area was ca. 23.5 m^2 ha^{-1}, and the aboveground volume averaged 145 m^3 ha^{-1}. The tree canopy ranged from 12.4 to 17.2 m in height (14.6 m average), with a stem diameter at breast height (DBH) ranging from 15.8 to 25.0 cm (19.9 cm average). The forest canopy had a mean depth of 8.9 m and a leaf area index (LAI, m^2; projected tree leaf area per m^2 of ground area) of 2.0; its seasonality has been described by *Hirata et al.* [2007]. The forest understory was predominantly buckler fern (*Dryopteris crassirhizoma* Nakai), with occasional bracken fern (*Dryopteris expansa* Fr.-Jenkins et Jermy) and Japanese spurge (*Pachysandra terminalis* Sieb. et Zucc.). The average biomass of the understory was 1.24 t ha^{-1}. Mean $\delta^{13}C$ values of the plant leaves were −29.7‰ PDB ("PDB" denotes that the isotope ratio is expressed on a PeeDee Belemnite (PDB) basis) for Japanese larch, −31.5‰ PDB for the mixed broadleaved species, and −28.2‰ PDB for buckler fern, respectively (T. Nakadai, ex NIES, Japan, personal communication; sampling was conducted in 2000–2001; large part of the overstory leaves were collected from lower and shaded part of the forest canopy).

[9] The soil at the site, a well-drained arenaceous soil developed from volcaniclastic sediment, was homogeneous and was classified as an immature Volcanogenous Regosol (Pumice). The nutrient-poor soil was weakly acidic (pH 5.0 to 6.0), with high porosity. The litter layer was 1 to 2 cm thick. Below the litter layer is a mat of organic layer between 5 and 10 cm thick and containing abundant fine roots; the next-deepest layer is fragments of porous pumice stone (0.5–3.0 cm in diameter) with some coarse roots. Scarcely any roots are found below a depth of 20 cm. Bulk organic matter collected from the soil surface at 25 plots in the forest revealed $\delta^{13}C$ values ranging from −27 to −29‰ PDB. There was a tendency common to all sampling plots that $\delta^{13}C$ of the organic matter increased as decomposition progressed from fresh plant material to litter and from litter to soil organic matter (T. Nakadai, ex NIES, Japan, personal communication; sampling was conducted in 2000–2001).

[10] The site was essentially flat, with a slope of 1–2°. The altitude ranged from 115 to 140 m above sea level. The site was characterized by a humid continental climate with cold winters and cool summers. The monthly mean air temperature and total monthly precipitation observed at the nearest weather station (Tomakomai weather station, 42°37.3′N, 141°32.8′E, located ca. 14 km south of the study site) are shown in Figure 1. The 30-year (1971–2000) mean annual precipitation was approximately 1229 mm; mean annual temperature was 7.5°C, with the mean monthly temperature ranging from 20.3°C in August to −4.1°C in January. Although precipitation is generally heavier in summer, all weather parameters exhibited significant year-to-year variation during the 3 years of our observation (2002, 2003, and 2004).

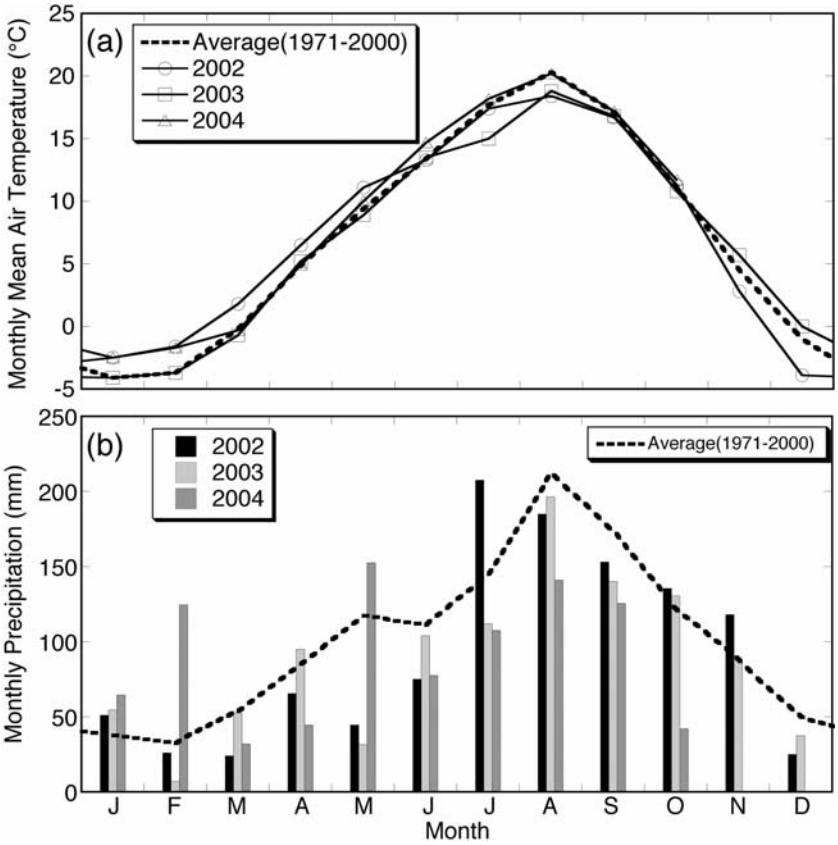

Figure 1. (a) Monthly mean air temperature and (b) total monthly precipitation observed at Tomakomai weather station during 3-year, 2002 to 2004. Dotted lines represent the 30-year (1971–2000) mean values.

[11] In this vegetation type at our study site, there was distinct seasonality in litterfall. Litterfall observed at 25 locations from 14 July (195 DOY) to 18 December (352 DOY) 2003 is shown in Figure 2. Litterfall was concentrated from October to November, and LAI decreased rapidly during this period [*Hirata et al.*, 2007]. This distinct seasonality in the input of fresh leaf litter to the soil system would lead to seasonal changes in the composition of the respiration substrates and, as a result, may influence the $\delta^{13}C$ of heterotrophic respiration.

[12] Our observations began in July 2002 and ended at the beginning of September 2004 because of extensive damage caused to the forest by typhoon 200418. In the typhoon event, more than 90% of trees were broken in the trunk part or pulled out by the root. The forest canopy had lost and the soil was disturbed seriously. The ecosystem in the site no longer has characteristics as a forest. Hence we abandoned further research at the site.

2.2. Sampling of Soil-Respired CO_2

[13] The main issues in the determination of the $\delta^{13}C$ of heterotrophic respiration are how to eliminate the influence of autotrophic (root) respiration from total soil CO_2 efflux and how to determine the $\delta^{13}C$ of soil CO_2 efflux without introducing measurement artifacts [e.g., *Högberg et al.*, 2005; *Ohlsson et al.*, 2005].

[14] In terms of the separation of heterotrophic respiration, the usability and limitations of the major approaches have been reviewed well by *Hanson et al.* [2000]. *Kuzyakov* [2006] distinguished source of soil CO_2 efflux in detail and evaluated the existing flux-partitioning approaches including root-exclusion, isotope-labeling, tree-girdling, component integration etc. All the approaches had intrinsic advantage and disadvantage, and no single, fully satisfactory flux partitioning method exist. In this study, we employed a variation of root exclusion technique called "root removal" coupled with chamber-based sampling because of its advantage in our main purpose, the long-term monitoring in natural environment.

[15] There are difficulties in the determination of the $\delta^{13}C$ of soil-respired CO_2 using chamber-based sampling. In the present paper, we have defined "soil-respired CO_2" as that which diffuses across the soil–atmosphere interface and "soil CO_2" as the CO_2 found within the soil. Hereafter, we have abbreviated the $\delta^{13}C$ of soil-respired CO_2 as $\delta^{13}C_{R\text{-}soil}$. The $\delta^{13}C_{R\text{-}soil}$ is controlled not only by CO_2 produced within the soil but also by diffusion. Under steady-state conditions, $\delta^{13}C_{R\text{-}soil}$ equals the integrated value of the $\delta^{13}C$ produced within the soil, but the $\delta^{13}C$ of soil CO_2 would be enriched by around 4.4‰ from the $\delta^{13}C_{R\text{-}soil}$ because of isotopic fractionation during diffusion [*Amundsen et al.*, 1998]. Chamber-based measurements can lead to physical disturbance of the CO_2 gradient at the soil–atmosphere interface and may thus produce artifacts in the estimation of soil CO_2 efflux [*Davidson et al.*, 2002] and thus, possibly in the estimation of $\delta^{13}C_{R\text{-}soil}$. In particular, any pressure

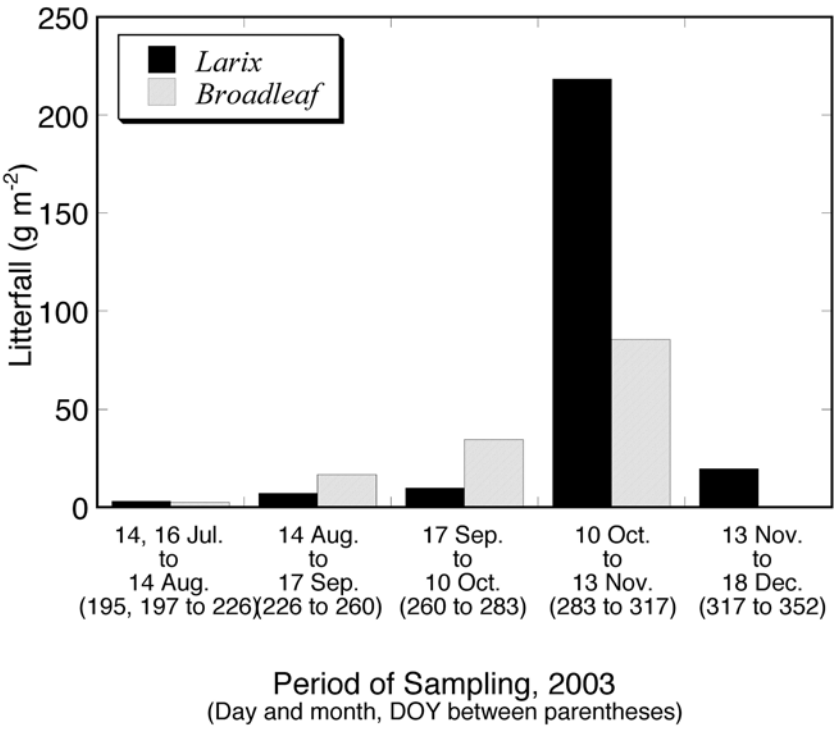

Figure 2. Litterfall observed at 25 locations from 14 July (195 DOY) to 18 December (352 DOY) 2003.

anomaly would cause mass flow of soil CO_2 with an anomalously enriched $\delta^{13}C$ value [*Högberg et al.*, 2005]. This effect would lead to artifact in the $\delta^{13}C_{R\text{-}soil}$ determination. In addition, alteration of $[CO_2]$ in the chamber would change the $[CO_2]$ gradient at the soil surface. It would potentially affect the ratio of $^{13}CO_2$ to $^{12}CO_2$ 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 $\delta^{13}C_{R\text{-}soil}$ 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.

[16] 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]. *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 CO_2. They reported that lowering of the initial CO_2 fraction induced significant enrichment (>4‰) in the $\delta^{13}C_{R\text{-}soil}$. As to the cause of the $\delta^{13}C_{R\text{-}soil}$ enrichment, the explanation was suggested that the initial lowering of the $[CO_2]$ caused a disturbance to the CO_2 diffusion process within the soil-chamber system. *Ohlsson et al.* [2005] also reported that the estimate of $\delta^{13}C_{R\text{-}soil}$ was not affected by the increase of $[CO_2]$ up to more than 2000 $\mu mol \cdot mol^{-1}$ for the non-flushed chamber though the apparent CO_2 efflux in the chamber was significantly declined. The result suggested that the acceptable $[CO_2]$ range for the $\delta^{13}C_{R\text{-}soil}$ determination was larger than that for the determination of soil CO_2 efflux.

[17] *Bowling et al.* [2003] and *McDowell et al.* [2004] employed static closed chambers coupled with a flask sampling system for the $\delta^{13}C_{R\text{-}soil}$ estimation with consideration for measurement artifacts related to pressure anomaly and to $[CO_2]$ enrichment in the chamber headspace. The main concept of their experimental configuration was similar to our sampling system used in this study.

[18] $\delta^{13}C_{R\text{-}soil}$ has been often estimated from vertical profiles of $[CO_2]$ and $\delta^{13}C$ of soil CO_2 [e.g., *Mortazavi et al.*, 2004; *Pendall et al.*, 2005]. This approach assumed that the respiratory end-member $\delta^{13}C$ value was constant with depth. The profiles of soil CO_2 and its $\delta^{13}C$ 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]. *Cisneros-Dozal et al.* [2006] suggested that leaf litter decomposition had large contributions to total soil CO_2 efflux during the growing season, and that the decomposition rate was highly sensitive to the soil water contents, from the experiment using radiocarbon. In seasonal vegetation types like deciduous forest or in tropical ecosystem, decomposition of surface litter would be a key component to determine the seasonality of soil CO_2 efflux and its $\delta^{13}C$. Therefore we considered that this soil-profile-based method was not appropriate for the objective of our study.

[19] In our field experiment, we employed a multichannel automated chamber system to continuously measure soil CO_2 efflux, combined with a flask sampling system optimized for collecting soil-respired CO_2 in this study. The chamber system had already been installed as part of a long-term soil-efflux monitoring study [*Liang et al.*, 2003]. The dimensions of each chamber were 0.9 × 0.9 m, with a

height of 0.5 m. The sampling area of each chamber was thus larger than that of previously described systems [e.g., *Flanagan et al.*, 1996; *Buchmann et al.*, 1997; *Bowling et al.*, 2003; *McDowell et al.*, 2004], and we predicted that this would help reduce the representation error that arises from small-scale spatial variability. The large volume, small vent, and slow movement of the pneumatically actuated lids effectively minimized pressure anomalies inside the chambers during their operation. The pressure fluctuation in the chamber was measured to be less than 0.22 Pa during regular CO_2 efflux measurements [*Liang et al.*, 2003].

[20] We designed the flask sampling system to collect air into a series of four flasks under positive pressure sequentially without introducing a pressure anomaly. For the flask sampling, the soil chamber was placed in series in a closed loop with a $Mg(ClO_4)_2$ (10–20 mesh, saturated with CO_2, GFS Chemicals, Powell, OH) water trap, a diaphragm pump (Model-MOA, GAST Mfg., Inc., Benton Harbor, MI, USA), an assembly of four glass flasks (750-mL, each with two vacuum stopcocks with a Viton® O-ring seal at both ends; Koshin Rikagaku Seisakusho, Tokyo, Japan), a back-pressure regulator (Model-6800AL, KOFLOC, Tokyo, Japan), and a flow-meter (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, flow-through or bypass the flasks, without stopping the airstream. The details of the flask sampling system are described by *Takahashi and Liang* [2008]. Inflow to and outflow from the soil chamber were balanced throughout a sampling operation using this structure. Sample lines (Dekabon 1300, 6-mm outer diameter, 10 m in length, Nitta-Moore, Tokyo, Japan) were located between the sampling system and the chambers. 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_2 efflux due to the covering of soil surface was unlikely. Efforts were made to avoid contaminating the atmosphere around the chambers with the CO_2 contained in human breath.

[21] We collected air samples using the following procedure. To avoid contaminating the air around the chambers with air imported from outside the observation site, all flasks were evacuated before measurements began. About 5 min before closing the chamber lid, all flask stopcocks were opened, and all solenoid valves were set to flow-through the flasks. 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 bypass mode 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 $L \cdot min^{-1}$ during the sampling. Overall collection times were shorter than 810 sec.

[22] We ascertained that fluctuation in pressure of chamber-headspace appeared during the switching of the flow-path in the sampling operation. Hence measurement artifacts related to pressure anomaly was supposedly negligible in our measurements. To assess the influence of enrichment of $[CO_2]$ in the chamber headspace, we tested consistency of the $[CO_2]$ increasing rate in unit time and linearity between $\delta^{13}C$ and $1/[CO_2]$ for all the sampling that we conducted. The maximum range of CO_2 in the chamber headspace from start to end of sampling was about 250 $\mu mol \cdot mol^{-1}$. Although the increasing rate of $[CO_2]$ significantly declined as the $[CO_2]$ enriched, we could not found evidence of the influence of the $[CO_2]$ enrichment on the linearity between $\delta^{13}C$ and $1/[CO_2]$ [*Takahashi and Liang*, 2008]. R^2 for the $\delta^{13}C$-vs-$1/[CO_2]$ relationship was never to be less than 0.995 and no systematic tendency was found in all the measurements. To our results, the estimates of the $\delta^{13}C_{R\text{-}soil}$ were rather insusceptible to the influence of the CO_2 enrichment in the chambers.

2.3. Root Exclusion

[23] To observe variations in the $\delta^{13}C$ of heterotrophic respiration under field conditions, we excluded roots by means of a method called "root removal" [*Hanson et al.*, 2000]. In June 2002, roots in five plots were carefully removed from the soil in 1.0 × 1.0 m areas to a depth of 30 cm, then the soil was returned to each pit with its original orientation preserved. Invasion of roots was prevented using vertical physical barriers made from polyvinylchloride boards on each side of the pits. The basal plane of the pits was not covered with these barriers. A lack of uniformity in belowground CO_2 production might have produced gradients in soil CO_2 and consequently caused lateral diffusion below the barriers. At this site, the soil layer was only 10 to 15 cm thick and the roots spread almost exclusively above a depth of 20 cm. Under those conditions, belowground CO_2 production would be concentrated within the soil above the basal plane of the barriers. The vertical CO_2 gradient above the basal plane would be steeper than the lateral gradient below the plane, lateral diffusion within the soil is unlikely to have been significant. Therefore, we have assumed that any CO_2 that invaded the chamber from outside the barrier as a result of lateral diffusion would have only a minor contribution to the CO_2 efflux observed in the root-exclusion plots.

[24] The possible disturbance of heterotrophic respiration caused by root removal was summarized by *Hanson et al.* [2000]. This technique would result in an initial flush of CO_2 out of the soil following disturbance. Time must pass for the increased CO_2 production rate to subside, and to allow time for the diffusion rates and production rates of CO_2 to come back to equilibrium. However, they argued that root exclusion studies are most useful if the measurements extend through a complete annual cycle. For a study with the objective of observing seasonal or longer-term variations in heterotrophic respiration, elimination of the contribution from turnover of the belowground litter caused by using this method was not possible, but we consider the resulting bias to be acceptably small.

[25] For the observations in 2002, it is possible that the impact of the physical disturbance created by removal of the roots had not yet disappeared. Hence, we caution readers

that the $\delta^{13}C$ seasonality observed in 2002 may be misleading. However, given the small magnitude of the disruption predicted by the results of *Hanson et al.* [2000] and the lack of any obvious difference between the 2002 response patterns and those in 2003 and 2004, we feel that the 2002 results are nonetheless useful in terms of the pattern, if not the magnitude, of the variation.

2.4. Laboratory Analysis

[26] We analyzed the $[CO_2]$ and the $\delta^{13}C$ of the CO_2 in the air samples collected at the study site in a laboratory of the National Institute for Environmental Studies. The $[CO_2]$ values in the samples were determined using a nondispersive IRGA (LI-6252, LI-COR, Lincoln, NE, USA) and were compared with our laboratory's CO_2 standard scale (the NIES95 scale) prepared using a gravimetric method. Our NIES95 scale was compared with a standard CO_2 scale provided by the Climate Monitoring and Diagnostics Laboratory of the National Oceanic and Atmospheric Administration (NOAA/CMDL) in 1996. Differences in $[CO_2]$ between the two laboratories were less than 0.12 μmol mol^{-1} for a range of values from 343 to 373 μmol mol^{-1}. The precision of the $[CO_2]$ analysis in the present study was estimated to be better than 0.10 μmol mol^{-1} based on replicated analysis and storing test for 1-week. After analyses of $[CO_2]$ and other gas components ($[CH_4]$, $[N_2O]$, $[CO]$, $[H_2]$, $[SF_6]$ and O_2/N_2 ratio), we performed cryogenic extraction of CO_2 for our isotopic measurement using a glass vacuum line. The principle of this extraction is similar to that described by *Vaughn et al.* [2004].

[27] Determination of $\delta^{13}C$ was also performed at the National Institute for Environmental Studies. The extracted CO_2 was introduced into an isotope-ratio mass spectrometer (IRMS; Delta-PLUS, Thermo Electron Co., Waltham, MA, USA) using variable-volume, dual-inlet devices. We corrected for the presence of N_2O in the sample CO_2 using measured $[N_2O]/[CO_2]$ values for each sample and a correction factor that accounted for differences in ionization efficiency between CO_2 and N_2O according to the concept of *Friedli and Siegenthaler* [1988].

[28] The carbon stable isotope ratio (delta notation) was defined as follows:

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

As for the stable isotope ratio of carbon, values were reported using the Vienna-PDB scale. The overall precision of the $\delta^{13}C$ analysis (including the CO_2 extraction process) was estimated to be better than 0.02‰ by replicated analysis and storing test (1 week). We also tested that $[CO_2]$ and $\delta^{13}C$ was not affected by passing through the $Mg(ClO_4)_4$ dryer as far as the deliquescence of the reagent did not occurs.

2.5. Determination of $\delta^{13}C$ of Soil-Respired CO_2 Using the Keeling Plot Approach

[29] To determine the $\delta^{13}C_{R\text{-}soil}$ value, we used a two-component simple mixing model called the "Keeling plot approach" [*Keeling*, 1958]. The usability and limitations of the Keeling plot approach were described in detail by *Pataki et al.* [2003]. In this study, we assumed that the relationship between $[CO_2]$ and $\delta^{13}C$ in the chambers was expressed by the following equation:

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

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 $\delta^{13}C_{R\text{-}soil}$ between the start and end of each sampling period, the intercept of the linear regression of $\delta^{13}C$ versus $1/[CO_2]$ observed in the chamber represents the $\delta^{13}C_{R\text{-}soil}$. We used Model I (ordinary least squares) regressions to determine $\delta^{13}C_{R\text{-}soil}$ according to the recommendation in *Zobitz et al.* [2006].

[30] As we mentioned above, the potential influence of the $[CO_2]$ enrichment was unavoidable in the chamber-based sampling sampling. While collecting samples with wider $[CO_2]$ range contributes to minimize standard error of $\delta^{13}C_{R\text{-}soil}$, it raises the risk of potential influence related to the $[CO_2]$ enrichment. To our results, the estimates of the $\delta^{13}C_{R\text{-}soil}$ were rather insusceptible to the influence of the $[CO_2]$ enrichment (up to c.a 250 mmol mol^{-1}, we tested) in the chamber as compared with the soil CO_2 efflux [*Takahashi and Liang*, 2008]. This result was consistent with the finding of *Ohlsson et al.* [2005].

[31] High-precision isotope measurement coupled with a sampling system optimized for soil respiration allowed us to capture the variability in $\delta^{13}C$ of soil-respired CO_2 under natural conditions.

3. Results and Discussion

3.1. Possible Causes of Representation Error With Chamber-Based Measurements

[32] To obtain valid estimates of isotopic disequilibrium between the atmosphere and the terrestrial biosphere and of seasonality in $\delta^{13}C_{R\text{-}soil}$, measurements of $\delta^{13}C_{R\text{-}soil}$ must be unbiased when compared with a representative value for appropriate spatial scales and timescales. "Snapshots" of these values obtained using a chamber-based sampling method would be representative for a spatial scale of approximately 0.8 m^2 and a timescale of several minutes. In terms of the objective spatial scale, we should attempt to estimate representative values for the stand-scale or larger. With a limited number of observations in chamber-based sampling, spatial heterogeneity in $\delta^{13}C_{R\text{-}soil}$ might lead to a serious representation error due to sampling bias. Large spatial variability in soil CO_2 efflux is a common phenomenon [e.g., *Liang et al.*, 2004; *Søe and Buchmann*, 2005] and it was predicted to be found also in the $\delta^{13}C_{R\text{-}soil}$. Before discussing the seasonal variation in $\delta^{13}C_{R\text{-}soil}$ or in the isotopic disequilibrium, we examine the short-term temporal variability and the spatial heterogeneity in $\delta^{13}C_{R\text{-}soil}$.

3.1.1. Short-Term Variations in $\delta^{13}C$ of Soil-Respired CO_2

[33] To illustrate the short-term variability in $\delta^{13}C_{R\text{-}soil}$, we brought forward the cases that the sampling was con-

ducted for two or more consecutive days. In Figure 3, we showed soil temperature, volumetric soil water content, soil CO_2 efflux and $\delta^{13}C_{R\text{-}soil}$ observed for the cases at a root-exclusion plot (chamber 3) and a non-root exclusion plot (chamber 10). In most cases ((b), (c), (d), (e), the second and the third data in (g), (h), (i) and (j)), the sampling was conducted in sequence, on an afternoon and then on next morning. The time periods corresponded nearly to that maximum and minimum of the soil temperature appeared in diurnal variation, respectively. The results from the root-exclusion plot (open circles) has remarkable feature that the change in the $\delta^{13}C_{R\text{-}soil}$ was inversely correlative to the change in the soil CO_2 efflux in all the cases except for (a) and (j). This correlative changes suggest that there is temperature-associated controlling factors on those short-term variations common to both the soil CO_2 efflux and the $\delta^{13}C_{R\text{-}soil}$.

[34] In second data points of case (a), $\delta^{13}C_{R\text{-}soil}$ at root-exclusion plot showed significant enrichment as the soil CO_2 efflux increased during a rapid rise of soil water content without significant changes of soil temperature. This anomalistic feature is most likely due to the extrusion of ^{13}C-enriched soil CO_2 by rainfall-induced change in soil water content. We cannot find reasonable explanation for the exceptional tendency in $\delta^{13}C_{R\text{-}soil}$ found in case (j).

[35] As for the short-term variations of soil CO_2 efflux in forest ecosystem, number of field-based studies has been published. *Tang et al.* [2003] reported that the soil CO_2 efflux exhibits significant diurnal variations associated with soil temperature. They suggested that the short-term variations in soil temperature might affect CO_2 efflux by changing the diffusion velocity near the air–soil interface and by the effects on microbial activity. *Gaumont-Guay et al.* [2006] investigated the seasonal and diurnal dependence of soil CO_2 efflux on soil temperature and soil water content based on the continuous half-hourly measurements of soil CO_2 efflux in a boreal forest. Their results suggested that the change of the vertical distribution of soil CO_2 production would have influence on temporal variations of soil CO_2 efflux. The change of the vertical distribution of soil CO_2 production was important factor to interpret the short-term variations of the $\delta^{13}C_{R\text{-}soil}$ observed in the root-exclusion plot. It has been commonly observed that $\delta^{13}C$ of soil organic matter become less depleted in greater depth in soil profile [e.g., *Ehleringer et al.*, 2000; *Bowling et al.*, 2002]. Soil in shallower depth exhibited diurnal temperature variation with greater amplitude. The change of temperature profile involve the change in vertical distribution of soil CO_2 production because decomposition rate of soil organic matter has significant temperature dependence, i.e., soil CO_2 production in the shallower depth is affected from greater soil temperature variation than in the deeper depth. Relative contribution of CO_2 produced in the shallower depth was to be greater in high-temperature time, and lesser in low-temperature time in the diurnal cycle. Assuming that the CO_2 produced in shallower depth had more depleted $\delta^{13}C$ than in greater depth, the $\delta^{13}C_{R\text{-}soil}$ should be more depleted in high-temperature time, and less depleted in low temperature-time. This hypothesis can provide a reasonable explanation for the observed short-term variation of the $\delta^{13}C_{R\text{-}soil}$ in the root-exclusion plot. But it should be noticed that we could not find quantitative relationship between the soil temperature gradient and the observed $\delta^{13}C_{R\text{-}soil}$ (we did not indicate in figure). The seasonal or long-term variations in the $\delta^{13}C_{R\text{-}soil}$ were not explicable solely by the effect of the change in the vertical distributions of soil CO_2 production.

[36] Temporal variations in soil CO_2 efflux are generally attributed also to change in soil water content [e.g., *Davidson et al.*, 1998; *Gaumont-Guay et al.*, 2006]. However, there was no evidence for seasonal drought at this site, because rainfall usually occurred once or twice a week. Abundant precipitation at our study site, coupled with good soil drainage, resulted in a volumetric soil water content, usually 30–40%, that was uniformly favorable to microbial activity throughout the growing season [*Liang et al.*, 2004]. Hence we considered that influence of soil water content was significant.

[37] Comparison of the result from the non-root-exclusion plot (crosses in the figures) with the root-exclusion plot (open circles) provided additional information on variation in $\delta^{13}C_{R\text{-}soil}$. The perturbations in CO_2 transport process at air-soil interface by changes in physical factors (i.e., temperature and rain pulse) should affect $\delta^{13}C_{R\text{-}soil}$ in a same sense for both the plots, with or without root-exclusion. In all cases except for (a), (b) and (g), tendency of the change in $\delta^{13}C_{R\text{-}soil}$ observed at the non-root-exclusion plot followed the tendency found at the root-exclusion plot in varying degrees. However, in cases (a), (b) and (g), $\delta^{13}C_{R\text{-}soil}$ at the non-root-exclusion plot showed tendency different from that at the root-exclusion plot. Those anomalous variations in the non-root-exclusion plot might reflect the change in photosynthetic isotope discrimination via root respired CO_2 because all the cases were in the growing season of the forest. *Tang et al.* [2005] found that tree photosynthesis modulates soil CO_2 efflux on a diurnal timescale. We suppose that higher supply of photosynthate from leaves to root in the season likely fluctuate the $\delta^{13}C_{R\text{-}soil}$.

[38] In our results, the range of short-term (<24 h) temporal changes in $\delta^{13}C_{R\text{-}soil}$ reached 0.73‰ in 5–6 December, 2002 (case (e)) and 0.65‰ in 13–14 Novem-

Figure 3. Short-term variations of soil temperature (uppermost panels), volumetric soil water content (second upper panels), soil CO_2 efflux (third upper panels) and $\delta_{13CR\text{-}soil}$ (lowermost panels) observed in a root-exclusion plot (chamber 3, indicated by open circles) and in a non-root-exclusion plot (chamber 10, indicated by crosses) in (a) 12–13 August (223–224 DOY) of 2002, (b) 21–22 August (232–233 DOY) of 2002, (c) 12–13 September (254–255 DOY) of 2002, (d) 25–26 October (297–298 DOY) of 2002, (e) 5–6 December (338–339 DOY) of 2002, (f) 17–20 June (167–170 DOY) of 2003, (g) 19–22 August (230–233 DOY) of 2003, (h)18–19 September (260–261 DOY) of 2003, (i) 23–24 October (295–296 DOY) of 2003, and (j) 13–14 November (316–317 DOY) of 2003, respectively. Open circles and crosses in the variations of soil temperature (uppermost panels) and of volumetric soil water content (second upper panels) indicates the time when sampling from the root-exclusion chamber and the non-root-exclusion chamber was conducted, respectively.

ber, 2003 (case (j)). It seems that the magnitude of the short-term variation become greater in colder period except for the case of rainfall (case (a)). We could not clarify the factors that determine the magnitude of the short-term change from existing information. The short-term variations in the $\delta^{13}C_{R\text{-}soil}$ provide representation error in analyses of long-term temporal variation and of spatial variation. To reduce the representation error, averaging of data observed

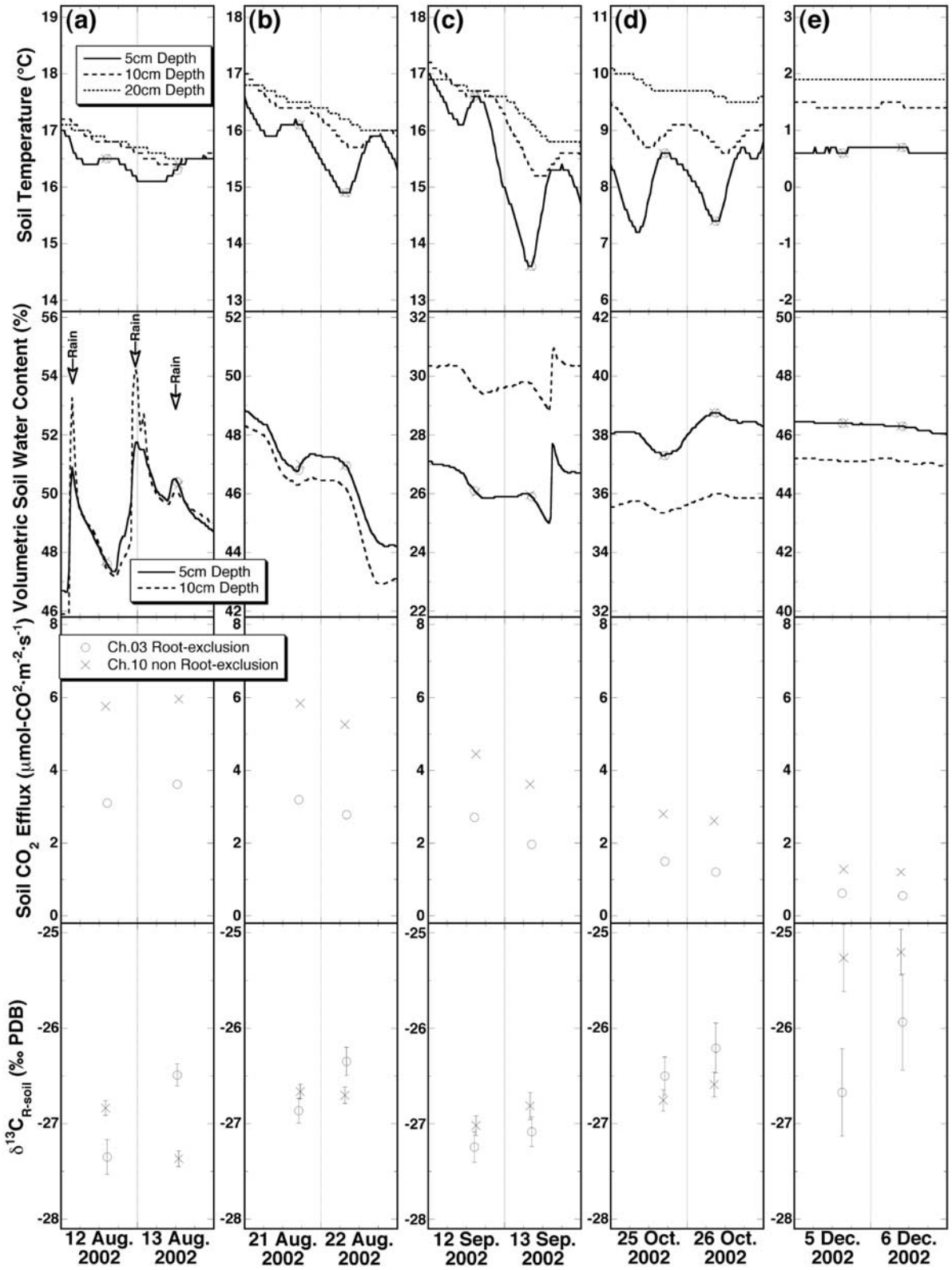

Figure 3

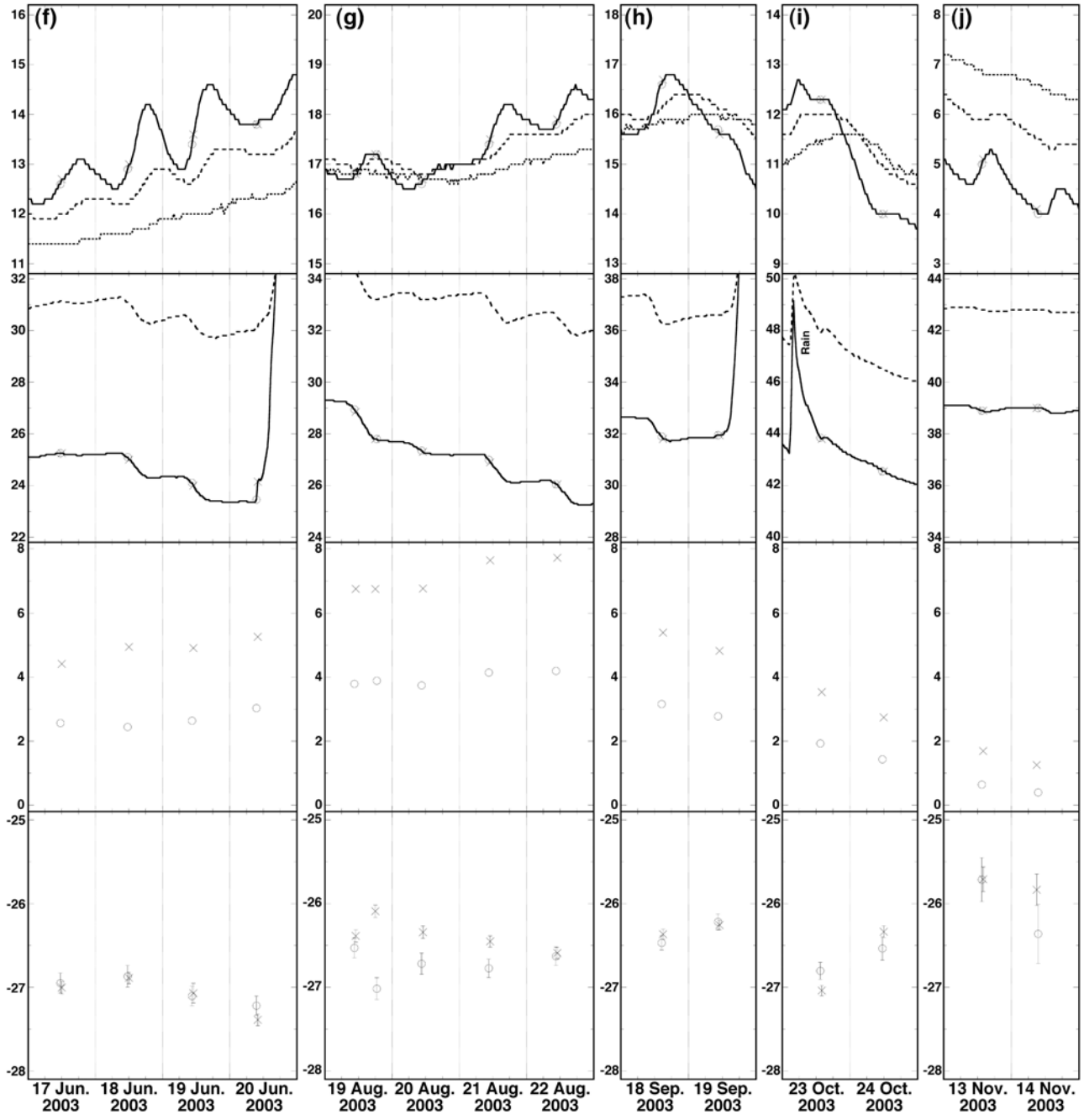

Figure 3. (continued)

in different time in the day would be effective means. Therefore, in the discussion of seasonal variations of the $\delta^{13}C_{R\text{-}soil}$ in later section, we used flux weighted mean values of the $\delta^{13}C_{R\text{-}soil}$ for the cases that multiple sampling was conducted in short period of time (i.e., the cases shown in Figure 3).

3.1.2. Spatial Variation in the $\delta^{13}C$ of Soil-Respired CO_2

[39] Although the study site was in an artificial forest and, consequently, was more uniform than a natural forest in many of the forest's properties (e.g., tree species, ages, and dimensions, as well as stand density), the forest floor was still heterogeneous at the spatial scale of the sampling chambers ($\sim$0.8 m^2). To test the spatial variability and representativeness of the $\delta^{13}C_{R\text{-}soil}$ values measured using the chambers, we collected samples using 10 chambers in different plots (five in the root-exclusion plots and five in non-root-exclusion plots) on 30 June and 27 August 2004 (Figure 4). The arrows in the figure denote the direction of the change from early to late summer. It was obvious that $\delta^{13}C_{R\text{-}soil}$ had high spatial variability in both the root-exclusion and non-root-exclusion groups at both times of year. The $\delta^{13}C$ value varied with the range larger than 2‰ in both treatment groups at both times of year. The significant spatial variability in the root-exclusion plots suggested

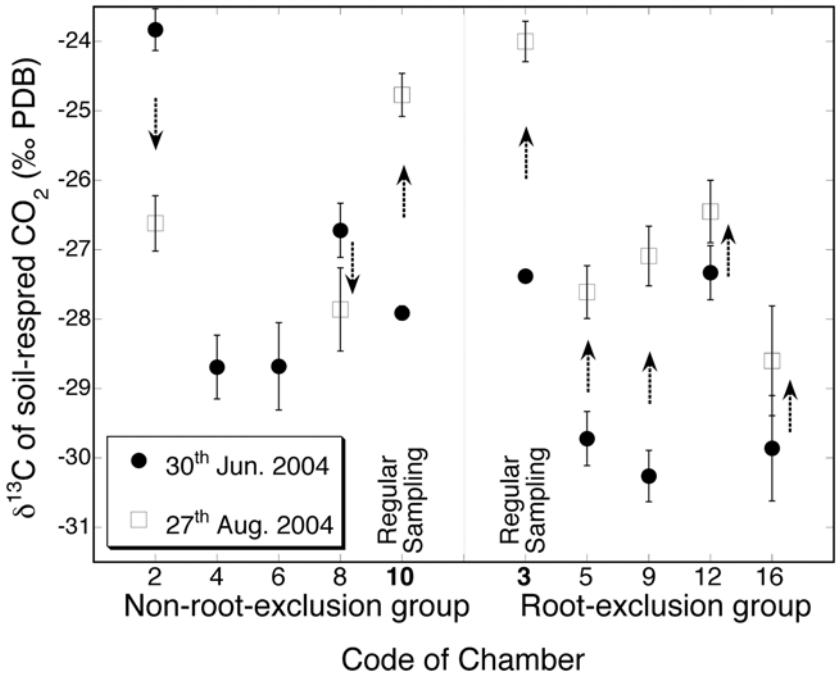

Figure 4. The $\delta^{13}C_{R\text{-}soil}$ observed at different location in the site. The five chambers in the left hand side were in non-root-exclusion plots and the five chambers in the right were in root-exclusion plots. Solid circles indicated that data observed in 30 June 2004, and open squares indicated that data observed in 27 August 2004. Error bars showed standard error of individual Keeling plot used in determination of $\delta^{13}C_{R\text{-}soil}$. In 27 August, samples collection from chambers 4 and 6 was failed because of troubles in the soil chamber and the sampling system. The arrows in the figure denote the direction of the change from early to late summer.

that some factors intrinsic to heterotrophic respiration must be responsible for the observed variability in $\delta^{13}C_{R\text{-}soil}$.

[40] A possible cause of this variability would be a lack of uniformity in the organic matter being decomposed at different locations. As mentioned previously, there were significant differences in $\delta^{13}C$ of the leaves of the different tree species at our study site. Variability in the relative contributions of these respiration substrates of different origin should be reflected in the heterogeneity of the mean $\delta^{13}C$ in the chambers. In addition, small-scale heterogeneity in the soil environment, such as differences in soil temperature and soil moisture, would also contribute to the spatial variability in $\delta^{13}C_{R\text{-}soil}$. Soil organic matter is composed of a range of compounds with different chemical forms, decomposition rates, and isotopic compositions [e.g., *Gleixner*, 2005]. This environmental heterogeneity might have caused the differences in the decomposition rates of individual substrates, which would consequently be reflected in the $\delta^{13}C_{R\text{-}soil}$ variability.

[41] *Davidson et al.* [2002] estimated confidence in the estimate of the mean soil CO_2 efflux by using equation described by *Folorunso and Rolston* [1984]:

$$n = \left[\frac{ts}{range/2}\right]^2 \tag{3}$$

where n is the sample number requirement, t the t-statistics for a given confidence level and degrees of freedom, s the standard deviation of the full population of measurements, an range the width of the desired interval about the full population mean in which a smaller sample mean is expected to fall. According to this equation, in our study site, ±20% precision with 95% confidence interval for the soil CO_2 efflux would be achieved by five sampling point with our chamber system [*Liang et al.*, 2004]. We calculated the number of sampling points required for determination of $\delta^{13}C_{R\text{-}soil}$ at the root-exclusion plot based on the data observed at 30 June 2004 (number of points was 5, mean was −28.91‰ and standard deviation was 1.43‰). The results demonstrate that 64, 254, and 6328 points are required to obtain an experimental mean within ±1‰, ±0.5‰ and ±0.1‰, respectively, with 95% confidence interval.

[42] At least at this study site, the representation error caused by the spatial variation in $\delta^{13}C_{R\text{-}soil}$ made it extremely difficult to determine the representative $\delta^{13}C_{R\text{-}soil}$ at the stand-scale or larger within an uncertainty of required level for validating modeled isotope disequilibrium (<1‰ as global mean) based on a feasible number of chamber-based measurements. Under these circumstances, we had to leave the observational validation of isotope disequilibrium between the soil and the atmosphere for future research.

[43] As for the soil CO_2 efflux, its significant spatial variability under natural environment are well known, but a lot remains to be established about factors that control the variation [e.g., *Davidson et al.*, 1998; *Buchmann*, 2000; *Xu and Qi*, 2001]. *Søe and Buchmann* [2005] investigated the spatial variations in soil CO_2 efflux and its controlling

factors in an unmanaged beech forest. Their results showed that spatial patterns of the soil CO_2 efflux was fairly stable throughout the growing season and was closely related to stand structure of the forest. This suggested that the spatial variation in the soil CO_2 efflux was largely controlled by direct and indirect influence from living plants. However, our results showed that the spatial pattern of the $\delta^{13}C_{R\text{-}soil}$ at non root-exclusion plots varies significantly between two observation periods in the growing season. This indicates that the spatial variations of the $\delta^{13}C_{R\text{-}soil}$ cannot be explicable directly from stand structure, even though root respiration had large contribution on the total soil CO_2 efflux. $\delta^{13}C$ of root respiration likely reflect the photosynthetic isotope discrimination of the plant [*Ekblad and Högberg*, 2001]. The photosynthetic isotope discrimination alters with environmental factors (availabilities of water, light, etc.) and physiological characteristics of plant leaves. In deciduous vegetation, adding to the short-term variations in environmental factors, the light availability for individual trees likely varies with seasonal changes of canopy structure. Furthermore, enzymatic CO_2 fixation efficiency of the leaves might have seasonal variation due to the plant phenology. Hence the $\delta^{13}C$ of root respired CO_2 likely have significant short-term and long-term variability specific to each individual locations. Consequently, its spatial pattern possibly has temporal variability.

[44] As for the situation in the absence of influence of living root, we found an interesting feature in the results shown in Figure 4. The value of $\delta^{13}C_{R\text{-}soil}$ tended to increase from July to August 2004 in all five plots in the absence of root respiration. In contrast, there was no common trend in the plots without root-exclusion. Even though the magnitude of this increase was anomalously large in the summer of 2004, a similar trend was found in the regular measurements in 2002 and 2003 at a root-exclusion plot (chamber 3; see section 3.2). Based on these results, we believe that it is possible to capture meaningful profiles of temporal variation in the $\delta^{13}C$ of heterotrophic respiration by means of regular, fixed-point observations of $\delta^{13}C_{R\text{-}soil}$ in the absence of root respiration.

3.2. Seasonal Variability in the $\delta^{13}C$ of Soil-Respired CO_2

[45] Figure 5 presents time series of $\delta^{13}C_{R\text{-}soil}$ observed by means of regular measurements at two fixed plots with root-exclusion (chamber 3) and without root-exclusion (chamber 10), along with the simultaneous changes in soil temperature and soil CO_2 efflux. The values of soil CO_2 efflux in this figure were calculated from the [CO2] in the flask samples. We use the CO_2 efflux values calculated from flask measurements, not from IRGA-measurement, to avoid introducing inconsistency due to the absence of some part of corresponding raw IRGA data recorded on-site has been uncollectible by some hardware accidents like lightning damage.

[46] In some sampling periods, we conducted multiple within 24 hours. We can find short-term changes in $\delta^{13}C_{R\text{-}soil}$ even in both plots with and without root-exclusion. The changes cannot be explicable only by contribution of root respired component. We supposed that the short-term variations in $\delta^{13}C_{R\text{-}soil}$ were associated with fluctuations in physical factors such as soil temperature and soil water content. Future work should address controls on the short-term $\delta^{13}C_{R\text{-}soil}$ variations. As we mentioned above, the range of short-term (<24 h) temporal changes in $\delta^{13}C_{R\text{-}soil}$ reached a maximum of more than 0.70‰. This magnitude in the short-term $\delta^{13}C_{R\text{-}soil}$ variations was not negligible compared with the seasonal $\delta^{13}C_{R\text{-}soil}$. However, the representation error caused by the short-term variation was effectively cancelled by the averaging of the data in many observation periods in our study. Hence we consider that the short-term $\delta^{13}C_{R\text{-}soil}$ variations would not affect decisively the interpretation of the seasonal variations.

[47] An important feature of this data was that the $\delta^{13}C_{R\text{-}soil}$ exhibited significant seasonal variability even in the absence of root respiration. The $\delta^{13}C_{R\text{-}soil}$ was generally lower in summer and higher in autumn and spring. In each year, the difference between the highest and lowest $\delta^{13}C_{R\text{-}soil}$ value was greater than 1‰. The amplitude of the seasonal variation in $\delta^{13}C_{R\text{-}soil}$ was thus larger than the sampling bias that resulted from short-term variations in $\delta^{13}C_{R\text{-}soil}$ ($\sim$0.50‰ in our experience, as described previously). In addition, the trend in the temporal variation in $\delta^{13}C_{R\text{-}soil}$ observed in the plots with and without root-exclusion (chamber 10) was similar. The variation in $\delta^{13}C_{R\text{-}soil}$ should thus be largely caused by changes in the $\delta^{13}C$ of the heterotrophic component of soil respiration. The lack of stand-scale representativeness of the $\delta^{13}C_{R\text{-}soil}$ values prevented us from quantifying the contribution of root (or heterotrophic) respiration to the overall $\delta^{13}C_{R\text{-}soil}$ variation using ^{13}C mass-balance equations based on the difference between chambers 3 (root exclusion) and 10 (no root exclusion). Hence, we do not discuss the contribution of root respiration to the variability in $\delta^{13}C_{R\text{-}soil}$ in this study.

[48] In general, the variation in soil CO_2 efflux is closely associated with changes in soil temperature and soil moisture. At our study site, soil CO_2 efflux was not regulated by a lack of soil moisture because there was frequent rainfall during the study period [*Liang et al.*, 2004]. Therefore, we will not discuss the influence of soil moisture. Instead, we tested the temperature dependencies of soil CO_2 efflux and of $\delta^{13}C_{R\text{-}soil}$, and their seasonal changes in the root-exclusion plot (chamber 3; Figure 6). Because the observations during 2002 and 2004 covered only half of the non-snow-covered period in these years, we have only shown the results during 2003. In Figures 6b and 6d, we used the data averaged for some sampling periods to reduce the representation error caused by short-term variations. The soil CO_2 efflux showed a clear exponential increase with increasing soil temperature. Soil CO_2 efflux during the progression of the seasons followed the same monotonic exponential curve, increasing as temperatures increased then decreasing again as temperatures decreased, with no evidence of hysteresis. In contrast, the relationship between $\delta^{13}C_{R\text{-}soil}$ and temperature followed no clear pattern, other than having an overall negative correlation. The sampling bias induced by the observed short-term variations in $\delta^{13}C_{R\text{-}soil}$ might be partially responsible for this seeming lack of a temperature dependency. However, the magnitude of the seasonal change in $\delta^{13}C_{R\text{-}soil}$ was still significant and meaningful. The value of $\delta^{13}C_{R\text{-}soil}$ formed a closed, ladle-shaped curve with the following seasonal course: (i) From spring (140 DOY) to summer (196 DOY), $\delta^{13}C_{R\text{-}soil}$ decreased, reaching its minimum value (196 DOY; Figure 6d) before

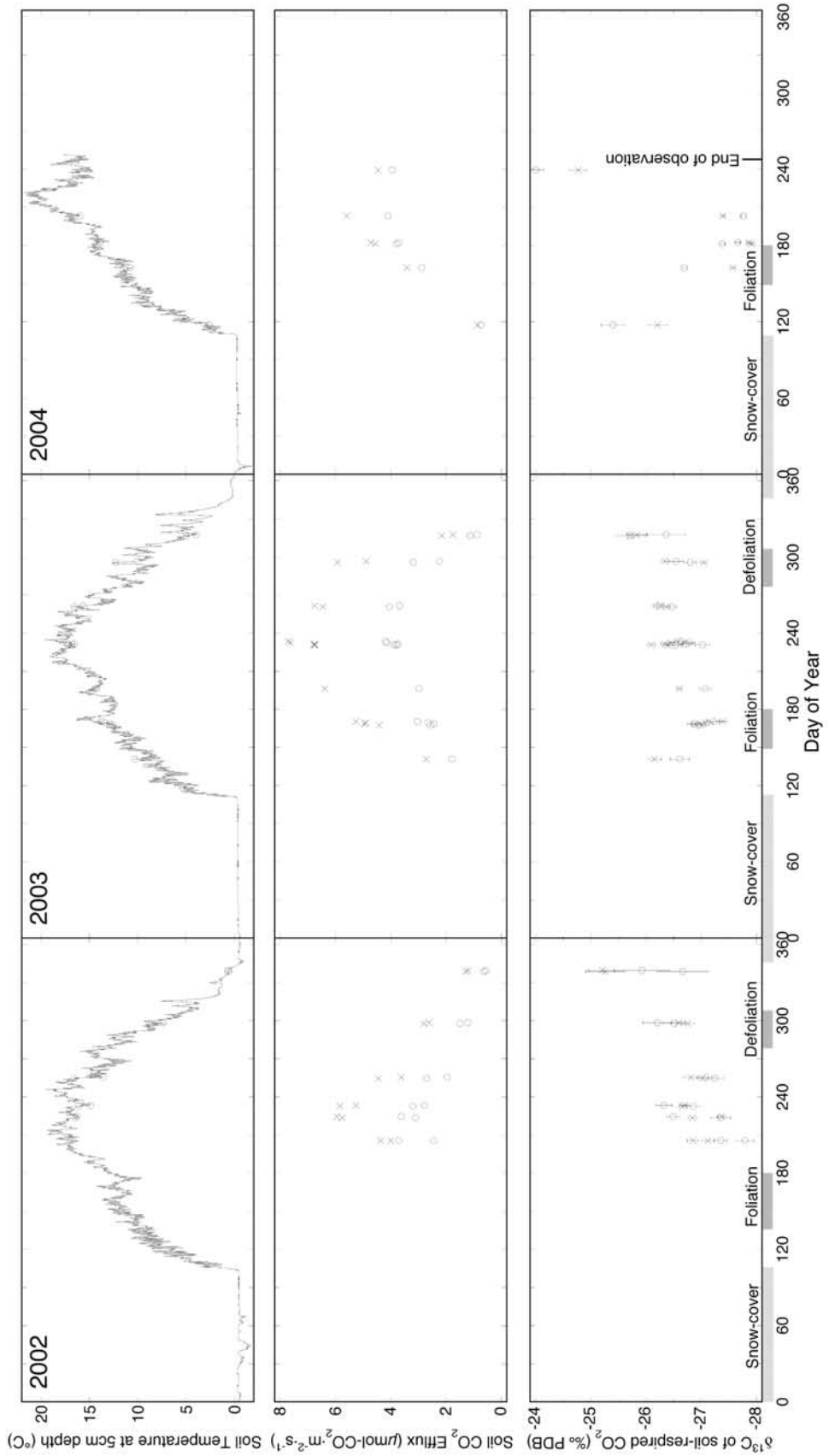

Figure 5. Time series of $\delta^{13}C_{R\text{-}soil}$ observed by means of regular measurements at two fixed plots with root-exclusion (chamber 3) and without root-exclusion (chamber 10), along with the simultaneous changes in soil temperature and soil CO_2 efflux.

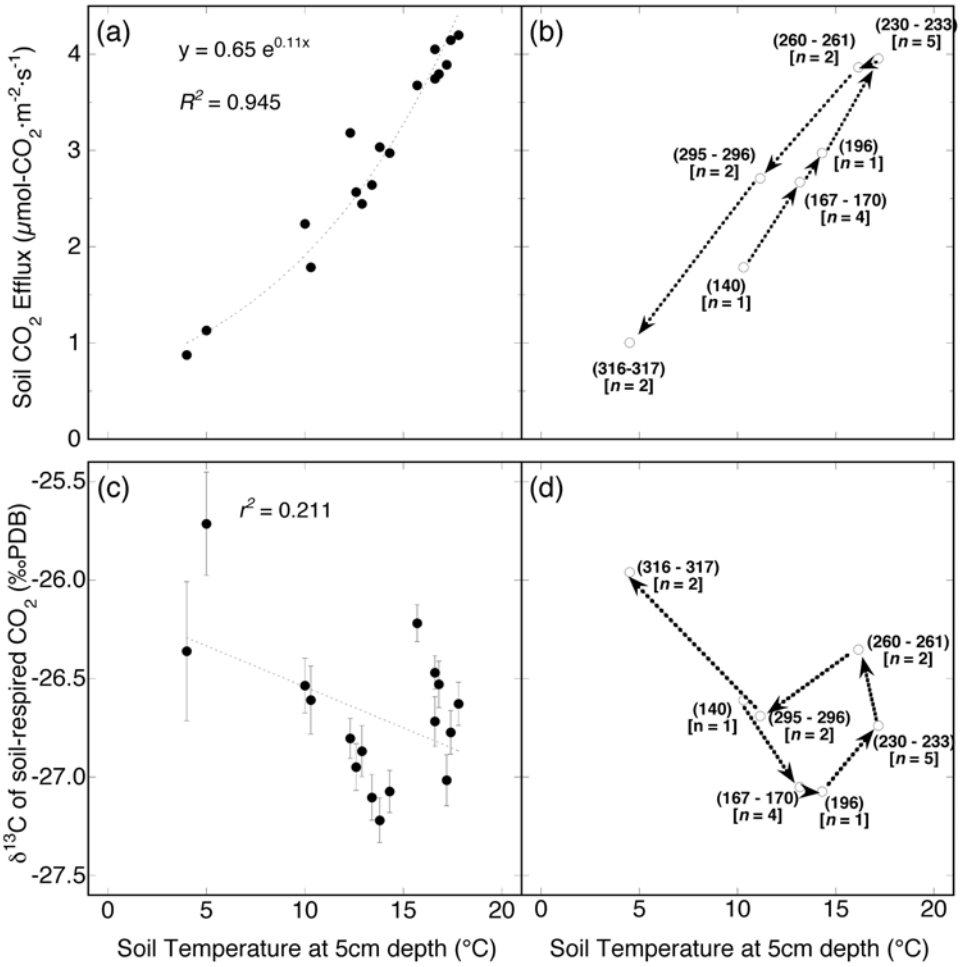

Figure 6. (a) Temperature relationship of the soil CO_2 efflux and (b) its time course. (c) Temperature relationship of the $\delta^{13}C_{R\text{-}soil}$ and (d) its time course. All results were obtained in the root-exclusion plot (chamber 3) in 2003. In figures for time course, (b) and (d), we used the data averaged for some sampling periods to reduce the representation error caused by short-term variations. Period of the averaging was shown between parentheses (in DOY) and number of data averaged was shown between brackets.

soil temperature reached its maximum value (230 to 233 DOY; Figure 10b). (ii) From early summer to early autumn (starting at 167 to 170 DOY and ending at 260 to 261 DOY), $\delta^{13}C_{R\text{-}soil}$ increased independently of the change in soil temperature. (iii) From autumn (260 to 261 DOY) to winter (316 to 317 DOY), $\delta^{13}C_{R\text{-}soil}$ initially decreased to a local minimum (at 295 to 296 DOY), then increased to its maximum (316 to 317 DOY) thereafter. This local minimum corresponded to the time of maximum litterfall (Figure 2). The decrease in $\delta^{13}C_{R\text{-}soil}$ from spring to summer was also observed in 2004 and the summer increase in $\delta^{13}C_{R\text{-}soil}$ was also found in 2002 and 2004 (Figure 5).

[49] These features suggest that the seasonality in $\delta^{13}C_{R\text{-}soil}$ could not be explained by means of a simple analogy with the soil CO_2 efflux. Changes in the quality of the decomposing soil organic matter could be a key factor in regulating the seasonal course of variations in $\delta^{13}C$ in heterotrophic respiration. Soil organic matter is composed of various carbon pools with different chemical forms, decomposition rates, and isotopic compositions [e.g., *Gleixner*, 2005]. In models [*Fung et al.*, 1997; *Randerson et al.*, 2002], the ^{13}C flux from heterotrophic respiration has been expressed as a composite value for these different compartments. *Andrews et al.* [1999] found the significant shift in $\delta^{13}C$ of CO_2 evolved from root-free soil with magnitude of about 2‰ in their 32-days incubation experiments. The shift was likely due to the decay of labile soil organic matter pool. In their study, root exudates and root detritus were supposed to be sources of the labile substrates. We consider that similar shift in $\delta^{13}C_{R\text{-}soil}$ might occur due to the decay of labile substrate in fresh leaf litter even in the absence of living roots.

[50] The seasonal variation in the $\delta^{13}C$ of heterotrophic respiration reflects the change in the relative composition of the flux components from the individual compartments. We hypothesize that the seasonality in the input and subsequent decomposition of leaf litter influenced this variation. The leaves of the larch and of the mixed broadleaved trees at our study site had a lower $\delta^{13}C$ value than the bulk organic matter in the surface soil, which suggests that the newly deposited leaf litter included a rapidly decomposing substrate with a lower $\delta^{13}C$ value and a slow-decomposing

substrate with a higher $\delta^{13}C$ value. The CO_2 that originated from the bulk leaf litter should gradually become ^{13}C-enriched as decomposition progresses.

3.3. Simulation of the $\delta^{13}C$ Seasonality Using a Simple Two-Compartment Model

[51] In this section, we illustrate the factors that contribute to the general characteristics of the observed seasonality in $\delta^{13}C$ of heterotrophic respiration. First, in general, the $\delta^{13}C$ of heterotrophic respiration was negatively correlated with the seasonal changes in soil temperature. Second, the seasonal course of the $\delta^{13}C$ of heterotrophic respiration formed a closed, ladle-shaped trajectory in the graph of $\delta^{13}C$ versus temperature (Figure 6d). We hypothesized that the former characteristic was mainly caused by differences in the temperature dependence of decomposition rates between the individual compartments of the carbon pool and that the latter characteristic was related to decay of the more labile ^{13}C-depleted substrate as decomposition progressed.

[52] We tested these hypotheses by means of a simulation that was based on a simple two-compartment (labile and slow-decaying organic matter) model. The main purpose of this test was to help us understand how the general characteristics of the $\delta^{13}C$ seasonality developed rather than to analyze them quantitatively. Assuming that the two individual compartments in the soil carbon system had unique temperature dependencies of their respective decomposition rates, we expressed the heterotrophic respiration (R_h) by a modified equation based on equation (4) in *Randerson et al.* [2002], as follows:

$$R_h = \sum_{i=1}^{n} k(i)C(i,t)Q_{10}(i)^{\frac{T(t)-T_{ref}}{10}}, \tag{4}$$

where $k(i)$ is the decomposition rate constant for pool i, C is the temporally varying carbon content of each pool, $Q_{10}(i)$ represents the change in decomposition rate per 10°C change in temperature [*Fang and Moncrieff*, 2001], $T(t)$ is the soil temperature at time t, and T_{ref} is the reference soil temperature on which Q_{10} is calculated. To simplify the equation, we substituted $R_{ref}(i, t)$ for $k(i)C(i, t)$.

$$R_h = \sum_{i=1}^{n} R_{ref}(i,t)Q_{10}(i)^{\frac{T(t)-T_{ref}}{10}}, \tag{5}$$

where R_{ref} represents the respiration rate at reference temperature T_{ref}. Since $k(i)$ is a constant, $R_{ref}(i, t)$ for each pool (i) is proportional to $C(i, t)$. Assuming that there is no isotopic fractionation during decomposition, the $\delta^{13}C$ of the CO_2 that originated from each compartment equals the $\delta^{13}C$ of the carbon in the compartment. Introducing $\delta^{13}C$ into the equation, we obtain:

$$\delta^{13}C_{R_h} \cdot R_h = \sum_{i=1}^{n} \delta^{13}C(i)R_{ref}(i,t)Q_{10}(i)^{\frac{T(t)-T_{ref}}{10}}, \tag{6}$$

where $\delta^{13}C_{Rh}$ is the $\delta^{13}C$ value for overall R_h and $\delta^{13}C(i)$ is the $\delta^{13}C$ of carbon in each compartment and the CO_2 that originated in the compartment. For simplicity, we have assumed that R_h consists of two compartments (i.e., $n = 2$ in equations (4), (5), and (6)): one with a larger contribution to soil CO_2 efflux and with a higher $\delta^{13}C$ (hereafter denoted by A), and another with a smaller contribution to the efflux and with a lower $\delta^{13}C$ (denoted by B). In this calculation, we used monthly mean soil temperature at a depth of 5 cm in 2003 as $T(t)$ in these equations and defined T_{ref} as 0°C. The time step used in this calculation was 1 month. In this situation, t corresponds to individual months. The respiratory flux from component i in month t would thus be expressed as follows:

$$R(i,t) = R_{ref}(i,t) \cdot Q_{10}(i)^{\frac{T(t)-T_{ref}}{10}}. \tag{7}$$

The sum of the CO_2 respired from both compartments during the course of a year was normalized to a value of 1, and $R_{ref}(i, t)$ for individual compartments was calculated with respect to this normalized value to give the relative contributions from compartments A and B to the annual flux of 80 and 20%, respectively. Thus:

$$\sum_{t=1}^{12} R(A,t) = 0.8 \tag{8a}$$

and

$$\sum_{t=1}^{12} R(B,t) = 0.2. \tag{8b}$$

[53] We set the annual mean flux value of the $\delta^{13}C$ of respired CO_2 to be −26.7‰ PDB based on the flux-weighted mean value of the $\delta^{13}C$ observed in 2003. Then the $\delta^{13}C(i)$ for compartment A was set to −26.0‰ PDB from the least depleted $\delta^{13}C$ value observed in 2003. Because this value should represent the situation that contribution from the compartment B was minimum. The value was the lower limit of the $\delta^{13}C$ assumable for the compartment A. Finally, the $\delta^{13}C(i)$ for compartment B was set to −29.5‰ PDB from mass balance. (The $\delta^{13}C(i)$ for compartment B was basically linked to the choice of the assumed relative contributions from each compartment in total CO_2 efflux. For example, if we set relative contribution from compartment B in the total CO_2 efflux to be 10% instead, the $\delta^{13}C(i)$ for compartment B was to be −33‰ PDB.) As we mentioned above, the leaves of larch, which was the dominant tree species of the site, had $\delta^{13}C$ value of about −29.7‰ PDB and degraded organic matter in the soil surface had less depleted $\delta^{13}C$ value than that. This involved that $\delta^{13}C$ of CO_2 evolved from degraded leaf litter was more depleted than the $\delta^{13}C$ of the living leaves, −29.7‰ PDB. Therefore the value of $\delta^{13}C(i)$ for compartment B, −29.5‰ PDB, is reasonable, although it is significantly lower than the most depleted $\delta^{13}C_{R\text{-}soil}$ value observed in 2003.

[54] First, we examined the influence of different temperature dependencies ($Q_{10}(i)$ in equations (4)–(6)) on the $\delta^{13}C$ seasonality and its temperature relationship under the hypothetical condition that the pool size ($C(i, t)$ in equation (4)) for the individual compartments remained constant over time. (Based on this condition, $R_{ref}(i, t)$ was also constant with time.) Assuming a constant size of the individual

compartments, which was the basic assumption in this test calculation, assumes an unrealistic condition in which carbon loss via respiration is compensated for by litter input at all times or that the carbon pool sizes for both compartments were infinitely larger than the carbon loss via respiration. Nonetheless, this approach permits a qualitative investigation of general trends in $\delta^{13}C$ seasonality. We compared the results obtained from three different combinations of temperature dependence (Figure 7): $Q_{10}(A) = Q_{10}(B)$, $Q_{10}(A) > Q_{10}(B)$, and $Q_{10}(A) < Q_{10}(B)$. We chose 1.5 for the $Q_{10}(A)$ value based on the rough estimates from soil CO_2 efflux observed in 2003 (Figure 6a). It should be noted that, because the number of the data points used in the Q_{10} determination is fairly small, the Q_{10} value probably contains large uncertainty. We set the $Q_{10}(B)$ value to be 1.0, 1.5 and 2.0, respectively, without concrete motive. It should be noted that factors other than magnitude relation of the Q_{10} values between the two compartments had no critical influence on conclusion of this calculation. When compartments A and B had the same Q_{10} value, $\delta^{13}C_{Rh}$ showed no variation with time even though R_h showed clear seasonal variation. This occurred because there was no temporal variation in the relative contribution of the two compartments to R_h. When Q_{10} was larger for compartment B than for compartment A, the larger contribution from compartment B decreased $\delta^{13}C_{Rh}$ during the summer. This suggests that the decreased $\delta^{13}C_{Rh}$ in summer could be explained by the different temperature dependence of decomposition in the two compartments. However, under the assumption of a constant carbon pool size, the temperature dependence of $\delta^{13}C_{Rh}$ showed a monotonic exponential change as a function of soil temperature that was dissimilar from the observational results.

[55] Second, to illustrate differences in seasonal trends in the temperature dependencies of R_h and $\delta^{13}C_{Rh}$, we introduced terms for the decay of the labile compartment into the model, because the decay of the carbon pool is an important factor that regulates the seasonality of R_h [*Randerson et al.*, 1996]. At our study site, leaf litter input into the soil carbon pool was concentrated in October and November. Hence, we made the basic assumption that the labile compartment with decreased $\delta^{13}C$ (compartment B in this calculation) was input into the soil system simultaneously as leaf litter at the beginning of November, and decomposed gradually during the following 12 months until the next input was supplied in the following November. In this situation, November represents the initial month ($t = 1$) in the model's 12-month sequence. The rate of decomposition was assumed to be regulated by the monthly mean soil temperature and by $Q_{10}(i)$, as follows:

$$C(B, t+1) = C(B, t) - R(B, t) \cdot \Delta t. \tag{9}$$

[56] To express the seasonal decrease in compartment size, we introduced the parameter D_d, which is defined as the ratio of the carbon decomposed during the 12-month period to the initial carbon abundance in compartment B just after the initial loading of leaf litter:

$$\sum_{t=1}^{12} \{R(B, t) \cdot \Delta t\} = D_d \times C(B, 1). \tag{10}$$

When $Q_{10}(B)$ and D_d are given, the ratio of $C(B, 1)$ to $C(A)$ can be identified uniquely. Consequently, values of $R(B, t)$ for the 12-month period can be determined. We can then calculate seasonal changes in R_h, the relative contribution of compartment B to R_h and $\delta^{13}C_{Rh}$, and the temperature dependencies of R_h and $\delta^{13}C_{Rh}$ for different values of D_d. When we assume the same Q_{10} values for both compartments, $Q_{10}(A) = Q_{10}(B)$, the seasonality of $\delta^{13}C_{Rh}$ is defined by the change in the ratio of the pool sizes of the two compartments. Under this condition, $\delta^{13}C_{Rh}$ decreased unidirectionally as the decay of compartment B progresses during the 12 months after the input of leaf litter. This suggests that the assumption of $Q_{10}(A) = Q_{10}(B)$ cannot explain the summer minimum for $\delta^{13}C_{Rh}$. Figure 8 shows the results of our simulation under three different set of values for $Q_{10}(A)$, $Q_{10}(B)$, and D_d: (i) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 2.0$, and $D_d = 0.50$ (solid squares); (ii) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 2.0$, and $D_d = 0.90$ (open circles); and (iii) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 5.0$, and $D_d = 0.90$ (crosses).

[57] Introduction of the decay in carbon pool size in compartment B led to a gap in the seasonal variation of the relative contribution of compartment B, R_h, and $\delta^{13}C_{Rh}$ between 2 months before and after the input of leaf litter. Compared with the relative contribution of compartment B and of $\delta^{13}C_{Rh}$, the gap in R_h was relatively small because the seasonality in R_h was bounded mainly by the contribution of compartment A, which was assumed to have constant size. When we compared cases (i) and (ii), the gap in $\delta^{13}C_{Rh}$ during the litter-input period was greater for the larger D_d value. In case (ii), the increasing trend from the litter-loading period dominated the temperature-associated variation in the $\delta^{13}C_{Rh}$ seasonality. The results of case (i) suggested that seasonal changes in the carbon pool size of compartment B explained the timing of the summer minimum of $\delta^{13}C_{Rh}$. The $\delta^{13}C_{Rh}$ reached its minimum in June, which was earlier than the period of maximum soil temperature (August). However, using the Q_{10} values we assumed in cases (i) and (ii), a prominent decrease of $\delta^{13}C_{Rh}$ and an increase in R_h appeared during the period of litter input. This change seemed to be larger than the observational results. We hypothesize that the low temperature during in the litter-input period suppressed the activity of the microbial community responsible for decomposition of the labile substrate (compartment B in this test calculation) under natural conditions. This situation can be expressed by using a greater temperature dependence for compartment B ($Q_{10}(B)$) in this calculation. The effect of different $Q_{10}(B)$ is illustrated by comparing cases (ii) and (iii). Using a $Q_{10}(B)$ value that was drastically larger than $Q_{10}(A)$ led to rapid decomposition of the carbon pool in compartment B during the high-temperature period. Consequently, this change suppressed the $\delta^{13}C_{Rh}$ decrease after the litter input and emphasized the summer $\delta^{13}C_{Rh}$ decrease. Consequently, the time series for $\delta^{13}C_{Rh}$ showed clear differences between the summer and other seasons, with a significantly large amplitude. In case (iii), the seasonal course of $\delta^{13}C_{Rh}$ in the $\delta^{13}C$-versus-temperature diagram (Figure 8e) formed a closed, ladle-shaped trajectory. This drastically high Q_{10} value may be caused by a phenological change in the population of microbes responsible for decomposition of the labile organic matter in the leaf litter. Nonuniformity in soil temperature also may have influence on the high Q_{10} value. The soil surface temperature likely had seasonal variation with greater magnitude than that in 5cm depth, which was used in

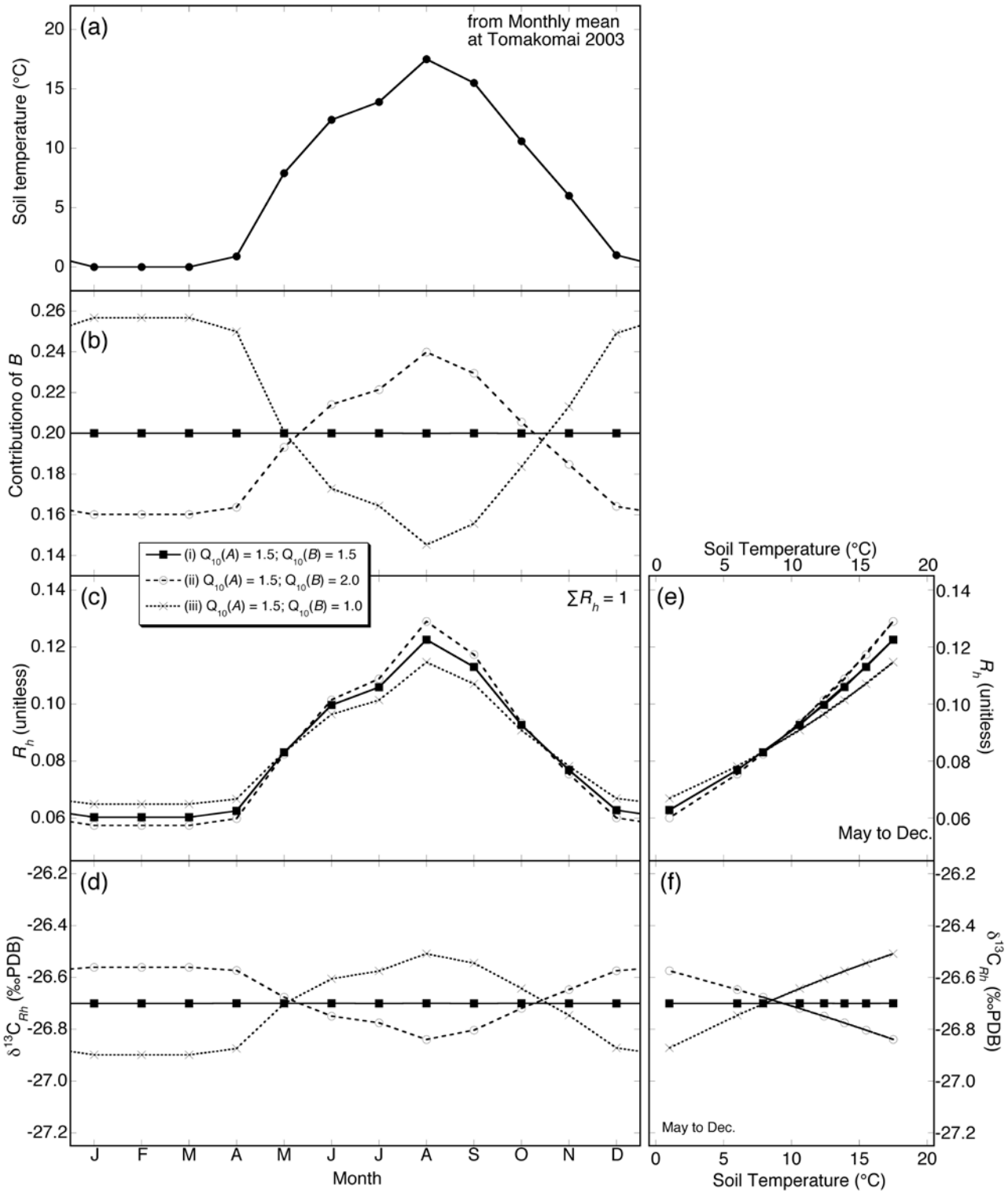

Figure 7. (a) Monthly mean soil temperature used in the model calculation. Simulated time variations in (b) relative contribution of compartment B on R_h, (c) R_h, and (d) $\delta^{13}C_{Rh}$. Temperature relationship of (e) R_h and (f) $\delta^{13}C_{Rh}$ from May to December. Solid squares, open circles and crosses indicate the results estimated on different combination of Q_{10} values, (i) $Q_{10}(A) = Q_{10}(B) = 1.5$, (ii) $Q_{10}(A) = 1.5$ and $Q_{10}(B) = 2.0$, and (iii) $Q_{10}(A) = 1.5$ and $Q_{10}(B) = 1.0$, respectively.

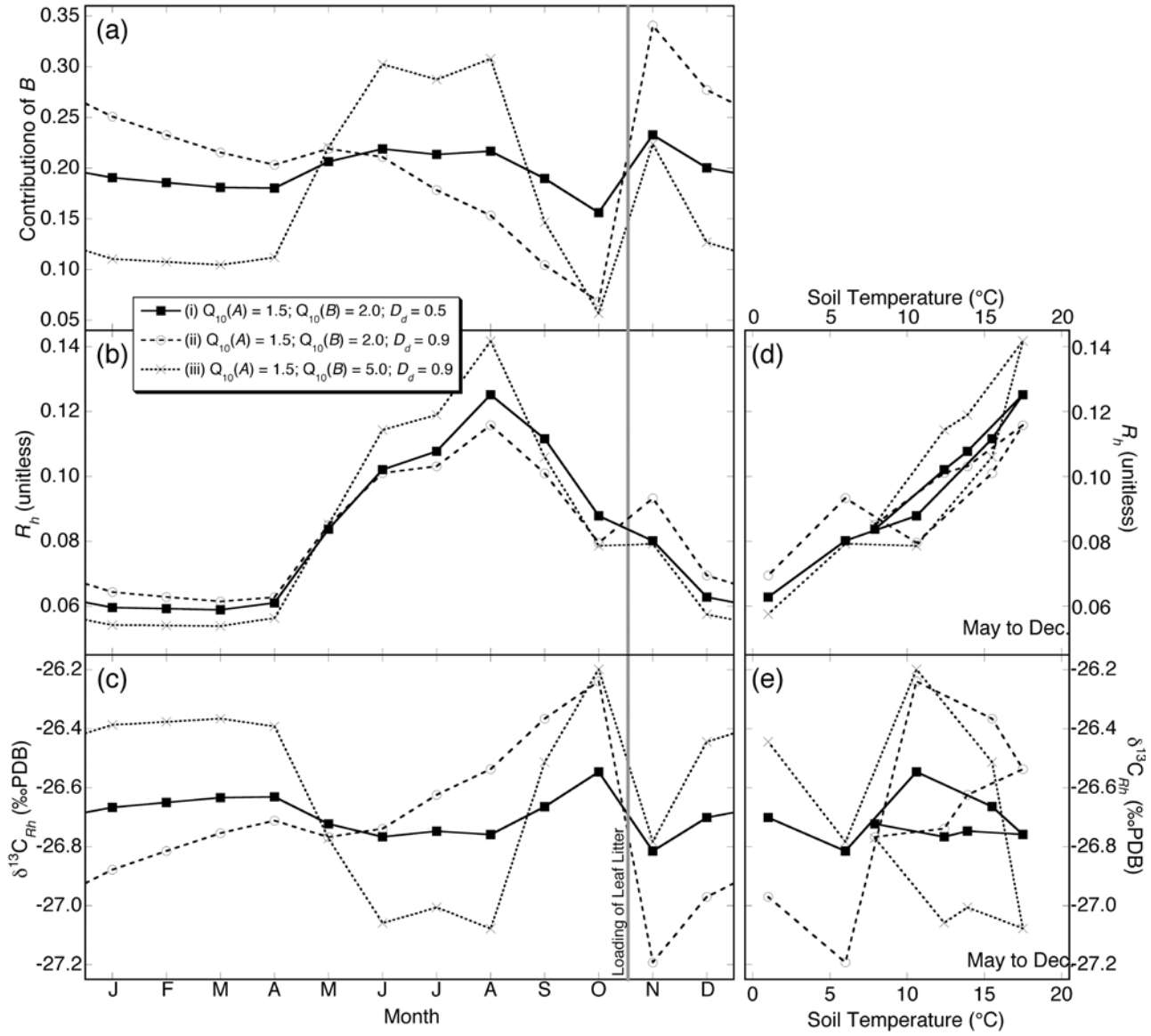

Figure 8. Simulated variations in (a) relative contribution of compartment-B on R_h, (b) R_h, and (c) $\delta^{13}C_{Rh}$. Temperature relationship of (d) R_h and (e) $\delta^{13}C_{Rh}$ from May to December. The difference in symbol indicates individual set of values used in the calculation; (i) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 2.0$ and $D_d = 0.50$ (indicated as solid square), (ii) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 2.0$ and $D_d = 0.90$ (open circles), and (iii) $Q_{10}(A) = 1.5$, $Q_{10}(B) = 5.0$ and $D_d = 0.90$ (crosses).

our simulation. Hence the labile component in the litter in the soil surface should be affected by greater seasonal temperature change under the natural environment than under the condition we assumed in the calculation.

[58] We notice here the influence of the choice of assumed values except for Q_{10} on the results. As we mentioned above, the $\delta^{13}C$ for compartment B is linked to the relative contributions of compartment B on the total CO_2 efflux in our determination procedure of the values. If we are to assume the higher $\delta^{13}C$ for compartment B, the relative contributions of the compartment B must be enlarged. Choice of the assumed values for the $\delta^{13}C$ and the relative contribution of compartment B likely affect the seasonal course of the soil CO_2 efflux, but not the course of the $\delta^{13}C_{Rh}$ significantly. This is because the effects from the lowered (or raised) $\delta^{13}C$ for compartment B and from the increased (or reduced) contribution of compartment B are compensative each other in the simulated seasonal course of the $\delta^{13}C_{Rh}$. On the other hand, the increasing of the contribution of compartment B poses a larger hysteresis in the seasonal course of temperature relationship of the soil CO_2 efflux according to the enlarged influence from the seasonal decay of pool size of compartment B.

[59] Difference in temperature dependence of the decomposition rate of organic matter among different soil carbon pools have been a topic of debate in the context of global warming. *Liski et al.* [1999] observed decomposition rate of old organic matter to be insensitive to temperature. Analysis of *Giardina and Ryan* [2000] suggested that the decomposition rate of organic carbon in forest mineral soil is not controlled by temperature limitation to microbial activity. Those field-based studies argued that older carbon pools

should have less temperature dependence in the decomposition. On the contrary, recent studies based on incubation experiment demonstrated that the temperature dependence would not vary with the age of carbon pools [*Fang et al.*, 2005; *Reichstein et al.*, 2005]. The focal point of those studies is to ascertain whether there exists significant difference in temperature dependence of the decomposition rate between in soil organic layer and in mineral soil. It should be noted that our discussion about the potential nonuniformity of the Q_{10} is directed to the processes in soil carbon pools with very short turnover time. Hence our findings in this study would hardly have important implications for the studies in the context of global warming because of discrepancy in subjected timescale.

[60] In summary, the simple two-compartment calculation illustrated that accounting for a combination of three key factors (a large intercompartment difference in $\delta^{13}C$, seasonal changes in the size of the labile compartment associated with input and decomposition of leaf litter, and a drastically greater Q_{10} value for the labile compartment) can simulate three notable characteristics found in our 2003 observations: (1) obvious seasonality that involves a summertime decrease in $\delta^{13}C_{Rh}$, (2) the appearance of the $\delta^{13}C_{Rh}$ minimum in advance of the soil temperature maximum, and (3) a local minimum of $\delta^{13}C_{Rh}$ during the period of concentrated litterfall.

[61] The ladle-shaped seasonal course of $\delta^{13}C_{Rh}$ in the diagram of $\delta^{13}C$ versus temperature (Figure 8e) was demonstrated by simulating the interplay of the abovementioned factors and appears to be a necessary result, even though it seemed odd at first glance. However, some points of difference remain between the simulated and observational results, such as the findings that the temperature dependency of R_h showed a distorted loop when we introduced a seasonal change in the pool size and that the obvious maximum for $\delta^{13}C_{Rh}$ appeared just before the period of litter input. The former observation suggests that the labile organic matter (compartment *B* in the simulation) contributed less (ca. 20%) to R_h than was assumed in the simulation. The second observation was likely modified by assuming a slower rate of decay of the labile organic matter than that assumed in the simulation (D_d = 0.90). If our hypothesis is correct that the labile organic matter, with highly decreased $\delta^{13}C$, has a high temperature dependence (Q_{10}), then the seasonal transition in $\delta^{13}C_{Rh}$ should be deeply influenced by anomalous soil temperatures during the high-temperature season because the decay of the labile substrate depends strongly on temperature. This hypothesis effectively explained the extreme increase of $\delta^{13}C_{R\text{-}soil}$ that we observed in August 2004. The soil temperature at a depth of 5 cm in 2004 was 2.2°C higher in July and 0.5°C higher in August than the temperatures in 2003. These high temperatures might have caused rapid depletion of the labile organic matter, consequently producing an anomalous increase in $\delta^{13}C_{R\text{-}soil}$ in late August 2004. It should be noted that the high temperature dependence of the decomposition rate of the labile substrate leads to significant year-to-year variation in the seasonality of $\delta^{13}C_{R\text{-}soil}$.

[62] We predicted that many factors of the seasonality in $\delta^{13}C_{R\text{-}soil}$ that we have discussed would be strongly associated with the strong seasonality of litterfall in this vegetation type (a deciduous needle-leaf forest). The seasonality in an evergreen needle-leaf forest, which is a dominant vegetation type in high-latitude forests of North America and northwestern Eurasia, can be expected to be more moderate because of the absence of this strong seasonality of litterfall in these forests. If the $\delta^{13}C_{R\text{-}soil}$ seasonality observed in the present study is broadly representative of this vegetation type, the seasonality might exert a considerable influence on the ^{13}C budget in high-latitude zones because this vegetation type dominates extensive areas of northeastern Eurasia.

4. Summary and Conclusions

[63] The results of this study indicate that combining high-precision measurements of $[CO_2]$ and $\delta^{13}C$ with a sampling protocol that minimizes the physical disturbance of the soil permits the precise determination of $\delta^{13}C$ of soil-respired CO_2 using chamber-based measurements with limited spatial and temporal scales ($\sim$1 m^2 and several minutes). However, representation errors (sampling bias) due to spatial heterogeneity in the $\delta^{13}C$ of soil-respired CO_2 made it difficult to apply the measured values to an analysis of larger spatial scales. Under the current limitations imposed by chamber-based sampling and IRMS-based measurements, we cannot offer a feasible solution that would reduce the representation error to the required level for validating modeled isotopic disequilibrium.

[64] Even though the chamber-based measurements involve this representation error, the fixed-point regular observations were nonetheless sufficiently useful that we were able to capture the seasonal variation in the $\delta^{13}C$ of soil-respired CO_2. During our 3-year observation of a deciduous needle-leaf forest, we found significant seasonal variation in the $\delta^{13}C$ of soil-respired CO_2 even with the influence of root respiration excluded. The $\delta^{13}C$ values decreased during the high-temperature season compared with its values during the low-temperature season. In each year, the amplitude of the seasonal change in $\delta^{13}C$ exceeded 1‰ and was remarkably larger than the prediction of previous models. In contrast to the soil CO_2 efflux, which exhibited a simple exponentially increasing temperature dependence, $\delta^{13}C$ showed a characteristic seasonal course in the diagram of $\delta^{13}C$ versus temperature. A simple simulation using a two-compartment model illustrated that the characteristic seasonal course of $\delta^{13}C$ could be qualitatively explained by accounting for the interplay of three factors: a large difference in $\delta^{13}C$ between the labile and resistant compartments of soil carbon, a significant seasonal change in the size of the labile carbon pool, and a higher temperature dependence of the decomposition rate in the labile compartment. The notable seasonality observed in $\delta^{13}C$ was probably associated with the characteristic litterfall pattern of this vegetation type.

Notation

$C(i, t)$	temporally varying carbon content of soil carbon pool i in the two-compartment model at time t.
$[CH_4]$	atmospheric CH_4 mixing ratio.
[CO]	atmospheric CO mixing ratio.
$[CO_2]$	atmospheric CO_2 mixing ratio.

$\delta^{13}C$ carbon stable isotope ratio (‰).
$\delta^{13}C_{RE}$ $\delta^{13}C$ of ecosystem-respired CO_2 (‰).
$\delta^{13}C_{R\text{-}soil}$ $\delta^{13}C$ of soil-respired CO_2 (‰).
$\delta^{13}C_{Rh}$ $\delta^{13}C$ value for R_h.
$\delta^{13}C_{RH}$ $\delta^{13}C$ of CO_2 from soil heterotrophic respiration (‰).
$\delta^{13}C(i)$ $\delta^{13}C$ of the carbon in compartment i and the CO_2 that originates from that comportment.
DBH stem diameter at breast height (cm).
D_d ratio of the carbon decomposed during a 12-month period to the initial carbon abundance in compartment B after the initial input of leaf litter.
DOY Day of year.
Δ_A photosynthetic isotope discrimination against $^{13}CO_2$.
F_{ba} respiratory CO_2 flux from the terrestrial ecosystem to the atmosphere.
F_{ap} CO_2 flux from the atmosphere to the terrestrial ecosystem; counter-flux of F_{ba}.
i the compartment number in the two-compartment model.
$[H_2]$ atmospheric H_2 mixing ratio.
$k(i)$ decomposition rate constant for carbon pool i in the two-compartment model.
LAI leaf area index (m^2).
$[N_2O]$ atmospheric N_2O mixing ratio.
$Q_{10}(i)$ change in decomposition rate per 10°C change in temperature.
R_h heterotrophic respiration.
$R_{ref}(i, t) = k(i)C(i, t)$; R_{ref} represents the respiration rate at reference temperature T_{ref}.
$R(i, t)$ respiratory flux from component i in month t.
$[SF_6]$ atmospheric SF_6 mixing ratio.
$T(t)$ soil temperature at time t.
T_{ref} reference soil temperature for which Q_{10} is calculated.

[65] **Acknowledgments.** We thank our colleagues at the Tomakomai Flux Research Site for their cooperation in this research. We also thank the members of the National Institute for Environmental Studies, who provided useful discussion, many suggestions, and their encouragement in this study. This research was partly funded by the Global Environmental Research Fund, Ministry of Environment, Japan. Finally we earnestly thank anonymous reviewer for helpful and constructive comment on this study.

References

Amundsen, R., L. Stern, T. Baisden, and Y. Wang (1998), The isotopic composition of soil and soil-respired CO_2, *Geoderma*, *82*, 83–114.

Andrews, J. A., K. G. Harrison, M. Roser, and W. H. Schlesinger (1999), Separation of root respiration from total soil respiration using carbon-13 labeling during free-air carbon dioxide enrichment (FACE), *Soil Sci. Soc. Am. J.*, *63*, 1429–1435.

Bousquet, P. (1999a), Inverse modeling of annual atmospheric CO_2 sources and sinks: 1. Method and control inversion, *J. Geophys. Res.*, *104*(D121), 26,161–26,178.

Bousquet, P. (1999b), Inverse modeling of annual atmospheric CO_2 sources and sinks: 2. Sensitivity study, *J. Geophys. Res.*, *104*(D121), 26,179–26,193.

Bowling, D. R., N. G. McDowell, B. J. Bond, B. E. Law, and J. R. Ehleringer (2002), ^{13}C content of ecosystem respiration is linked to precipitation and vapor pressure deficit, *Oecologia*, *131*, 113–124.

Bowling, D. R., D. E. Pataki, and J. R. Ehleringer (2003), Critical evaluation of micrometeorological methods for measuring ecosystem–atmosphere isotopic exchange of CO_2, *Agric. For. Meteorol.*, *3118*, 1–21.

Buchmann, N. (2000), Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands, *Soil Biol. Biochem.*, *32*, 1625–1635.

Buchmann, N., J.-M. Guehl, T. S. Barigah, and J. R. Ehleringer (1997), Interseasonal comparison of CO_2 concentrations, isotopic composition, and carbon dynamics in an Amazonian rainforest (French Guiana), *Oecologia*, *110*, 120–131.

Ciais, P., P. P. Tans, M. Trolier, J. W. C. White, and R. J. Francey (1995a), A large northern hemispheric terrestrial CO_2 sink indicated by the $^{13}C/^{12}C$ ratio of atmospheric CO_2, *Science*, *269*, 1098–1102.

Ciais, P., P. P. Tans, J. W. C. White, M. Trolier, R. J. Francey, J. A. Berry, D. A. Randall, P. J. Sellers, J. G. Collatz, and D. S. Shimel (1995b), Partitioning of ocean and land uptake of CO_2 inferred by $\delta^{13}C$ measurements from the NOAA/CMDL global air sampling network, *J. Geophys. Res.*, *100*(D3), 5051–5070.

Ciais, P., P. Friedlingstein, D. S. Shimel, and P. P. Tans (1999), A global calculation of the $\delta^{13}C$ of soil respired carbon: Implications for the biospheric uptake of anthropogenic CO_2, *Global Biogeochem. Cycles*, *13*(2), 519–530.

Ciais, P., M. Cuntz, M. Scholze, F. Mouillot, P. Peylin, and V. Gitz (2005), Remarks on the use of ^{13}C and ^{18}O isotopes in atmospheric CO_2 to quantify biospheric carbon fluxes, in *Stable Isotopes and Biosphere–Atmosphere Interactions: Processes and Biological Controls*, edited by L. B. Flanagan, J. R. Ehleringer, and D. E. Pataki, pp. 235–267, Elsevier, San Diego, Calif.

Cisneros-Dozal, L. M., S. Trumbore, and P. J. Hanson (2006), Partitioning sources of soil-respired CO_2 and their seasonal variation using a unique radiocarbon tracer, *Global Change Biol.*, *12*, 194–204.

Davidson, E. A., E. Belk, and R. D. Boone (1998), Soil water content and temperature as independent or confounded factors controlling soil respiration in a temperate mixed hardwood forest, *Global Change Biol.*, *4*, 217–227.

Davidson, E. A., K. Savage, L. V. Verchot, and R. Navarro (2002), Minimizing artifacts and biases in chamber-based measurements of soil respiration, *Agric. For. Meteorol.*, *113*, 21–37.

Ehleringer, J. R., N. Buchmann, and L. B. Flanagan (2000), Carbon isotope ratios in belowground processes, *Ecol. Appl.*, *10*, 412–422.

Ekblad, A., and P. Högberg (2001), Natural abundance of ^{13}C in CO_2 respired from forest soils reveals speed of link between tree photosynthesis and root respiration, *Oecologia*, *127*, 305–308.

Ekblad, A., B. Boström, A. Holm, and D. Comstedt (2005), Forest soil respiration rate and $\delta^{13}C$ is regulated by recent above ground weather conditions, *Oecologia*, *143*, 136–142.

Fang, C., and J. B. Moncrieff (2001), The dependence of soil CO_2 efflux on temperature, *Soil Biol. Biochem.*, *33*, 155–165.

Fang, C., P. Smith, J. B. Moncrieff, and J. U. Smith (2005), Similar response of labile and resistant soil organic matter pools to changes in temperature, *Nature*, *433*, 57–59.

Flanagan, L. B., J. R. Brooks, G. T. Varney, S. C. Berry, and J. R. Ehleringer (1996), Carbon isotope discrimination during photosynthesis and the isotope ratio of respired CO_2 in boreal forest ecosystem, *Global Biogeochem. Cycles*, *10*(4), 629–640.

Folorunso, O. A., and D. E. Rolston (1984), Spatial variability of field-measured denitrification gas fluxes and soil properties, *Soil Sci. Soc. Am. J.*, *48*, 1213–1219.

Francey, R. J., P. P. Tans, C. E. Allison, I. G. Enting, J. W. C. White, and M. Trolier (1995), Changes in oceanic and terrestrial carbon uptake since 1982, *Nature*, *373*, 326–330.

Friedli, H., and U. Siegenthaler (1988), Influence of N_2O on isotopic analyses in CO_2 and mass-spectrometric determination of N_2O in air samples, *Tellus*, *40B*, 129–133.

Fung, I. Y., et al. (1997), Carbon 13 exchanges between the atmosphere and biosphere, *Global Biogeochem. Cycles*, *11*(4), 507–533.

Gaumont-Guay, D., T. A. Black, T. J. Griffis, A. G. Barr, R. S. Jassal, and Z. Nesic (2006), Interpreting the dependence of soil respiration on soil temperature and water content in a boreal aspen stand, *Agric. For. Meteorol.*, *140*, 220–235.

Giardina, C. P., and M. G. Ryan (2000), Evidence that decomposition rates of organic matter in mineral soil do not vary with temperature, *Nature*, *404*, 858–861.

Gleixner, G. (2005), Stable isotope composition of soil organic matter, in *Stable Isotopes and Biosphere–Atmosphere Interactions: Processes and*

Biological Controls, edited by L. B. Flanagan, J. R. Ehleringer, and D. E. Pataki, pp. 29–46, Elsevier, San Diego, Calif.

Hanson, P. J., N. T. Edwards, C. T. Garten, and J. A. Andrews (2000), Separating root and soil microbial contributions to soil respiration: A review of methods and observations, *Biogeochemistry*, *48*, 115–146.

Heimann, M., and C. D. Keeling (1989), A three-dimensional model of atmospheric CO_2 transport based on observed wind: 2. Model description and simulated tracer experiments, in *Aspects of Climate Variability in the Pacific and the Western Americas*, edited by D. H. Peterson, pp. 237–276, AGU, Washington, D. C.

Hirata, R., T. Hirano, N. Saigusa, Y. Fujinuma, K. Inukai, Y. Kitamori, Y. Takahashi, and S. Yamamoto (2007), Seasonal and interannual variations in carbon dioxide exchange of a temperate larch forest, *Agric. For. Meteorol.*, *147*, 110–124.

Högberg, P., A. Ekblad, A. Nordgren, A. H. Plamboeck, A. Ohlsson, B. Bhupinderpal-Singh, and M. N. Högberg (2005), Factors determining the ^{13}C abundance of soil-respired CO_2 in boreal forests, in *Stable Isotopes and Biosphere–Atmosphere Interactions: Processes and Biological Controls*, edited by L. B. Flanagan, J. R. Ehleringer, and D. E. Pataki, pp. 47–68, Elsevier, San Diego, Calif.

Keeling, C. D. (1958), The concentration and isotopic abundances of atmospheric carbon dioxide in rural areas, *Geochim. Cosmochim. Acta*, *13*, 322–334.

Keeling, C. D. (1995), Interannual extremes in the rate of rise of atmospheric carbon dioxide since 1980, *Nature*, *375*, 666–670.

Kuzyakov, Y. (2006), Sources of CO_2 efflux from soil and review of partitioning methods, *Soil Biol. Biochem.*, *38*, 425–448.

Liang, N., G. Inoue, and Y. Fujinuma (2003), A multichannel automated chamber system for continuous measurement of forest soil CO_2 efflux, *Tree Physiol.*, *23*, 825–832.

Liang, N., T. Nakadai, T. Hirano, L. Qu, T. Koike, Y. Fujinuma, and G. Inoue (2004), In-situ comparison of four approaches to estimating soil CO_2 efflux in a northern larch (*Larix kaempferi* Sarg.) forest, *Agric. For. Meteorol.*, *123*, 97–117.

Liski, J., H. Ilvesniemi, A. Mäkelä, and C. J. Westman (1999), CO_2 emissions from soil in response to climatic warming are overestimated- the decomposition of old soil organic matter is tolerant of temperature, *Ambio*, *28*, 171–174.

McDowell, N. G., D. R. Bowling, B. J. Bond, J. Irvine, B. E. Law, P. Anthoni, and J. R. Ehleringer (2004), Response of the carbon isotopic content of ecosystem, leaf, and soil respiration to meteorological and physiological driving factors in a *Pinus ponderosa* ecosystem, *Global Biogeochem. Cycles*, *18*, GB1013, doi:10.1029/2003GB002049.

Mortazavi, B., J. L. Prater, and J. P. Chanton (2004), A field-based method for simultaneous measurements of the $\delta^{18}O$ and $\delta^{13}C$ of soil CO_2 efflux, *Biogeosciences*, *1*, 1–9.

Ohlsson, K. E., A. Ohlsson, B. Bhupinderpal-Singh, S. Holm, A. Nordgren, L. Lövdahla, and P. Högberg (2005), Uncertainties in static closed chamber measurements of the carbon isotopic ratio of soil-respired CO_2, *Soil Biol. Biochem.*, *37*, 2273–2276.

Pataki, D. E., J. R. Ehleringer, L. B. Flanagan, D. Yakir, D. R. Bowling, C. J. Still, N. Buchmann, J. O. Kaplan, and J. A. Berry (2003), The application and interpretation of Keeling plots in terrestrial carbon cycle research, *Global Biogeochem. Cycles*, *17*(1), 1022, doi:10.1029/2001GB001850.

Pendall, E., J. Y. King, A. R. Moser, J. Morgan, and D. M. Högberg (2005), Stable isotope constraints on net ecosystem production under elevated CO_2, in *Stable Isotopes and Biosphere–Atmosphere Interactions: Processes and Biological Controls*, edited by L. B. Flanagan, J. R. Ehleringer, and D. E. Pataki, pp. 182–198, Elsevier, San Diego, Calif.

Randerson, J. T., M. V. Thompson, C. M. Malmström, C. B. Field, and I. Y. Fung (1996), Substrate limitations for heterotrophs: Implications for models that estimate the seasonal cycle of atmospheric CO_2, *Global Biogeochem. Cycles*, *10*, 585–602.

Randerson, J. T., et al. (2002), Carbon isotope discrimination of arctic and boreal biomes inferred from remote atmospheric measurements and a biosphere–atmosphere model, *Global Biogeochem. Cycles*, *16*(3), 1028, doi:10.1029/2001GB001435.

Rayner, P. J., I. G. Enting, R. J. Francey, and R. Langenfelds (1999), Reconstructing the recent carbon cycle from atmospheric CO_2, $\delta^{13}C$ and O_2/N_2 observations, *Tellus*, *51B*, 213–232.

Reichstein, M. J.-A., E. Subke, A. C. Angeli, and J. D. Tenhunen (2005), Does the temperature sensitivity of decomposition of soil organic matter depend upon water content, soil horizon, or incubation time?, *Global Change Biol.*, *11*, 1754–1767.

Scholze, M., J. O. Kaplan, W. Knorr, and M. Heimann (2003), Climate and interannual variability of the atmosphere-biosphere $^{13}CO_2$ flux, *Geophys. Res. Lett.*, *30*(2), 1097, doi:10.1029/2002GL015631.

Søe, A. R. B., and N. Buchmann (2005), Spatial and temporal variations in soil respiration in relation to stand structure and soil parameters in an unmanaged beech forest, *Tree Physiol.*, *25*, 1427–1436.

Takahashi, Y., and N. Liang (2008), Development of chamber-based sampling technique for determination of carbon stable isotope ratio of soil respired CO_2 and evaluation of influence of CO_2 enrichment in chamber headspace, *Geochem. J.*, *41*, 493–500.

Tang, J., D. D. Baldocchi, Y. Qi, and L. Xu (2003), Assessing soil CO_2 efflux using continuous measurements of CO_2 within the soil profile with small solid-state sensors, *Agric. For. Meteorol.*, *118*, 207–220.

Tang, J., D. D. Baldocchi, and L. Xu (2005), Tree photosynthesis modulates soil respiration on a diurnal timescale, *Global Change Biol.*, *11*, 1298–1304.

Tans, P. P., J. A. Berry, and R. F. Keeling (1993), Oceanic $^{13}C/^{12}C$ observations: A new window on ocean CO_2 uptake, *Global Biogeochem. Cycles*, *7*, 353–368.

Vaughn, B. H., J. Miller, D. F. Ferretti, and J. W. C. White (2004), Stable isotope measurements of atmospheric CO_2 and CH_4, in *Handbook of Stable Isotope Analytical Techniques*, edited by P. A. de Groot, pp. 272–304, Elsevier, Amsterdam.

Xu, M., and Y. Qi (2001), Soil-surface CO_2 efflux and its spatial and temporal variations in a young ponderosa pine plantation in northern California, *Global Change Biol.*, *7*, 667–677.

Zobitz, J. M., J. P. Keener, H. Schnyder, and D. R. Bowling (2006), Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research, *Agric. For. Meteorol.*, *136*, 56–75.

Y. Fujinuma, R. Hirata, N. Liang, T. Machida, and Y. Takahashi, Center for Global Environmental Research, National Institute for Environmental Studies, 16-2 Onogawa, Tsukuba, Ibaraki 305-8506, Japan. (yoshiyu@nies.go.jp)