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Agricultural and Forest Meteorology 128 (2005) 67–79
AGRICULTURAL AND FOREST METEOROLOGY
www.elsevier.com/locate/agrformet

An empirical method of measuring CO_2 recycling by isotopic enrichment of respired CO_2

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Received 13 April 2004; accepted 30 August 2004

Abstract

An empirical method to measure respiratory CO_2 recycling using a fast growing agricultural cover crop as a model system was tested and compared with a theoretical method which uses a variation of the Keeling plot. Both methods gave values which were high and similar to each other. The theoretical method gave a value of respiratory based CO_2 recycling of 0.41, while the empirical method gave a value of 0.49. Therefore close to half of the respired CO_2 is refixed during daytime photosynthesis in this densely planted cover crop. Refixation of respired CO_2 during the day should lead to an isotopic enrichment of the remaining respired CO_2 leaving the canopy of the cover crop. Therefore, calculations of gross respiration and photosynthesis using isotopic mass balance equations that do not take this isotopic fractionation into account could be in error. We tested this premise by using isotopic mass balance equations to estimate average gross photosynthesis and respiration in this cover crop under two scenarios: (1) no recycling and (2) recycling of respired CO_2. Values of gross photosynthesis and respiration were unrealistically low when it was assumed that no recycling occurs. On the other hand, realistic values similar to previous publications were observed when recycling was taken into account.

Keywords: Keeling plots; Recycling; Gross photosynthesis; Gross respiration; Carbon isotope ratios; Isotopic mass balance equations

1. Introduction

The atmospheric CO_2 concentration is increasing at a rapid rate. Isotopic evidence indicates that this increase is mostly caused by anthropogenic input of CO_2 from the burning of fossil fuel among other sources (Houghton, 1991; Schimel et al., 2001). The rate of increase in CO_2 concentration, however, is less than that predicted on the basis of the quantities of anthropogenic input, leading to the conclusion that there is a carbon sink in the biosphere (Schimel et al., 2001). Stable isotope data indicate that this sink is of a terrestrial origin (Ciais et al., 1995). For this and other

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doi:10.1016/j.agrformet.2004.08.007

reasons there has been a strong interest in documenting net ecosystem exchange (NEE) of various terrestrial ecosystems. Networks of eddy covariance towers such as Ameriflux, Euroflux and Asiaflux are extensively distributed through out the globe. Although in some cases eddy covariance data have to be filtered, they can be used to accurately predict trends in NEE. Net ecosystem exchange, however, is the net result of two major CO_2 exchange processes: gross photosynthesis (P) and gross respiration (R). Eddy covariance techniques are not capable of partitioning NEE into these two components. Gross photosynthesis and respiration, although not completely independent of each other, are driven by different climatic parameters. Gross photosynthesis and respiration will respond differently to factors such as temperature, soil pH, light levels and mineral nutrition. It has been reported in the literature, for example, that variation in NEE of European forest ecosystems is primarily determined by variation in the gross respiration component (Valentini et al., 2000). One of the consequences of the increase in greenhouse gases in the biosphere is climatic change such as temperature and cloud cover. In order to be able to predict changes in NEE of ecosystems with climate change it is therefore important to understand how gross respiration and photosynthesis contribute to NEE of each ecosystem (Yakir and Sternberg, 2000).

There are four possible ways of partitioning NEE into its respective components. The first way is to measure soil, root, stem and leaf respiration through out the period of NEE measurements and scale these measurements up to gross respiration (Ryan et al., 1996; Law et al., 1999). Gross respiration is then added to NEE to give gross photosynthesis. This method is labor intensive. Further, the measure of respiration for some of the components, such as stem and leaf may be difficult particularly during the day. The second method involves locating eddy covariance instrumentation below the canopy to directly measure soil respiration (Baldocchi et al., 2000). This method can be problematic in interpreting eddy covariance data because of low turbulence below the canopy (Raupach et al., 1992). The third method involves correlating NEE versus temperature (Goulden et al., 1996). The fourth method uses isotopic mass balance principles to solve for gross respiration and photosynthesis (Yakir and Wang, 1996; Yakir and Sternberg, 2000; Bowling et al., 2001; Ogée et al., 2003). Implicit in the application of the isotopic mass balance methods used by the above investigators is the assumption that respired CO_2 recycling in ecosystems is negligible. Here we hypothesize that the calculation of gross respiration and photosynthesis by the isotopic mass balance method without taking recycling into account can lead to errors in the estimation of these parameters. These errors will be particularly large when recycling is high. The measurement of respired CO_2 recycling in itself presents a challenge. Currently there are two definitions of recycling at the ecosystem level (Schleser and Jayasekera, 1985; Sternberg, 1989, 1997; Lloyd et al., 1996, 1997; Sternberg and DeAngelis, 2002) and any study considering recycling must precisely define the type of recycling it is referring to. One recycling index, called here the photosynthetic based recycling index, refers to the flux of respired CO_2 fixed by photosynthesis relative to the total photosynthetic flux (Schleser and Jayasekera, 1985). The other recycling index, called here the respiratory based recycling index, refers to the flux of respired CO_2 fixed by photosynthesis relative to the total respiratory flux (Sternberg, 1989). Of particular interest in this study is the respiratory based recycling index. Other than a steady-state model equation to calculate this recycling index (Sternberg, 1989), we know of no other methods to estimate respired CO_2 recycling.

In this study we first measure recycling in an agricultural cover crop stand using a previously developed steady-state model equation (Sternberg, 1989). Second, we develop an empirical method to determine recycling in the same cover crop stand and compare its value with the theoretically derived value. This empirical method involves the artificial ^{13}C labeling of respired CO_2 in a treatment plot and comparing the isotopic composition of its respired CO_2 and biomass with those of a control plot. Finally, we demonstrate that ignoring recycling in the solution of isotopic mass balance equations to solve for gross respiration and photosynthesis can lead to large errors.

The advantage of an agricultural cover crop as a simple model ecosystem is that it can be completely harvested having its total biomass carbon reflecting integrated NEE over the period of growth. Further, the isotopic composition of total biomass integrates the isotopic flux over the period of growth.

2. Theory

2.1. Determining respiratory based recycling with a modified Keeling equation

The relationship between concentration and $\delta^{13}C$ values for mixtures of respired and atmospheric CO_2 having distinct carbon isotopic composition follows the Keeling equation (Keeling, 1961):

$$\delta_E = \frac{[CO_2]_A}{[CO_2]_E}(\delta_A - \delta_R) + \delta_R, \quad (1)$$

where the subscripts E, A, and R represent either the $\delta^{13}C$ value or concentration of: the ambient CO_2 where the mixture is found (such as vegetation canopies), the atmospheric source of CO_2 (defined here as the isotopic ratio of CO_2 from above the canopy of the cover crop) and the respired CO_2, respectively. Therefore, when $\delta^{13}C$ values of ambient CO_2 composed of a mixture of atmospheric and respired CO_2 is plotted against the inverse of the ambient CO_2 concentration, a linear relationship is observed having the $\delta^{13}C$ value of the respired CO_2 as its intercept (Keeling, 1961). The Keeling equation is only applicable when there is no photosynthetic uptake of ambient CO_2 which could lower the CO_2 concentration and alter its isotopic composition. For this reason data for Keeling plots are collected only at night when no photosynthesis occurs (Pataki et al., 2003). There can be several problems in the use of Keeling plots to interpret $\delta^{13}C$ values of respired CO_2, particularly for situations where there is a small range of CO_2 concentrations (Pataki et al., 2003), which was encountered during this study in the treatment plot. We therefore took another approach to derive the $\delta^{13}C$ value of respired CO_2. We rearranged the Keeling equation above to:

$$\delta_R = \frac{([CO_2]_E\,\delta_E - [CO_2]_{AN}\,\delta_{AN})}{([CO_2]_E - [CO_2]_{AN})}. \quad (2)$$

For each night of measurement we used the sample from 3 to 5 m height with the least concentration to represent isotopic composition (δ_{AN}) and concentration $[CO_2]_{AN}$ of night-time atmospheric CO_2. We then solved for the isotopic composition of respired CO_2 for each ambient CO_2 sample with Eq. (2) and averaged all values to derive the average $\delta^{13}C$ value of respired CO_2 ($\bar{\delta}_R$). We propose that by this method errors will be reduced in the estimate of the $\delta^{13}C$ value of respired CO_2 by fixing the $\delta^{13}C$ value of atmospheric CO_2. Furthermore, the average isotope ratio of the respired CO_2 will be based on several estimates rather than a single intercept.

In the case where photosynthesis is present, a modified Keeling equation has been derived for steady-state conditions (Sternberg, 1989):

$$\delta_E = \frac{[CO_2]_{AD}}{[CO_2]_E}\{(\bar{\delta}_{AD} - \bar{\delta}_R)(1 - \Phi_R)\} + \bar{\delta}_R + \Delta_L\,\Phi_R, \quad (3)$$

where Φ_R and Δ_L are the respiratory based recycling index and the photosynthetic isotopic discrimination at the leaf level, respectively. Since a large difference in atmospheric CO_2 concentration and isotopic composition was observed for CO_2 above the canopy between night and day we use the average daytime values ($[CO_2]_{AD}$ and $\bar{\delta}_{AD}$, respectively), which were collected at 5 m height, in the above equation. The photosynthetic isotopic discrimination at the leaf level (Δ_L) pertains to the isotopic discrimination of the leaf during CO_2 uptake relative to the isotopic composition of canopy CO_2.

We calculated Φ_R only in the control plot because the estimate of ($\bar{\delta}_R$) for this plot had a lower error compared to that in the treatment plot. Average leaf level photosynthetic fractionation was calculated with the following equation (Farquhar et al., 1982):

$$\bar{\Delta}_L = 4.4 + \left(22.6\frac{\bar{C}_i}{\bar{C}_{CH}}\right), \quad (4)$$

where $\bar{C}_i$ and $\bar{C}_{CH}$ represent the average internal CO_2 concentration and the external CO_2 concentration in the chamber during photosynthetic measurements, respectively. Since average values were used in Eq. (4) the discrimination factor calculated here represents an average discrimination factor ($\bar{\Delta}_L$). We assume that because this was an agricultural crop grown under constant conditions and a relatively short period there is little difference in the discrimination during the experimental period. Having the calculated values of $\bar{\Delta}_L$, $\bar{\delta}_R$, $[CO_2]_{AD}$, $\bar{\delta}_{AD}$, and several measurements of daytime value of δ_E at the canopy level (1.0, 1.5 and 2 m height), and their respective concentration ($[CO_2]_E$) we solved for Φ_R with Eq. (3) for each measurement of daytime ambient CO_2 and calculated the average value ($\bar{\Phi}_R$). Implicit in this solution is the assumption that the respired CO_2 during the day at its

source has the same isotopic composition as that measured at night.

2.2. Determining respiratory based recycling with the isotopic composition of biomass

If the growth conditions in a control and treatment plot only differ in the isotopic composition of respired CO_2, then the $\delta^{13}C$ values of biomass produced by these two plots can be expressed by the following two equations:

$$B\,\delta_B = B_A(\bar{\delta}_{AD} - \bar{\Delta}_C) + B_R(\bar{\delta}_R - \bar{\Delta}_C), \quad (5)$$

$$B\,\delta'_B = B_A(\bar{\delta}_{AD} - \bar{\Delta}_C) + B_R(\bar{\delta}'_R - \bar{\Delta}_C). \quad (6)$$

The total amount of biomass produced in each plot (B) should be similar (assumed here to be the same) and composed of biomass produced from atmospheric CO_2 fixation (B_A) and respired CO_2 fixation (B_R). The isotopic composition of these two sources (atmospheric and respired CO_2), when incorporated into biomass, will be modified by the average canopy discrimination ($\bar{\Delta}_C$). This discrimination factor differs from the previous leaf level discrimination because it also takes into account the fractionation associated with the diffusion of CO_2 between the canopy airspace and the site of carboxylation (Ogée et al., 2003; Bowling et al., 2003). In this experimental set up $\bar{\Delta}_C$ need not be solved for since it is eliminated in the solution of the simultaneous Eqs. (5) and (6). The primed symbols represent the isotopic composition of the labeled respired CO_2 ($\bar{\delta}'_R$) and that of the biomass ($\bar{\delta}'_B$) in the treatment plot. The two equations above solved simultaneously yield the integrated photosynthetic based recycling index:

$$\bar{\Phi}_P = \frac{B_R}{B} = \frac{\delta'_B - \delta_B}{\bar{\delta}'_R - \bar{\delta}_R}, \quad (7)$$

which is the total amount of biomass fixed from respiration (B_R) relative to the total amount of fixed biomass (B). If there is no recycling (i.e. no uptake of labeled respired CO_2), there will be no difference between the isotopic composition of biomass from treatment and control plots (i.e.$\delta'_B = \delta_B$). To solve Eq. (7) we determined the average isotopic values of respiratory CO_2 with Eq. (2) in the control and treatment plots using data pooled from all sample dates. The isotopic composition of biomass was determined by summing the product of the isotope ratios of root, stem and leaf tissue with their respective proportions and carbon content for samples collected within the treatment area.

The integrated respiratory based recycling index($\bar{\Phi}_R$), which is the total amount of respired carbon fixed into biomass relative to that given off by respiration, was calculated by the following equation:

$$\bar{\Phi}_R = \frac{\bar{\Phi}_P\, B\, \%C}{R_d\, 12}, \quad (8)$$

where %C is the proportion of carbon in biomass and R_d is the total amount of daytime carbon respired during the experimental period of 63 days (mol/m^2). The respiratory based recycling index shown in Eq. (8), was solved for both plots. To solve Eq. (8), we used the %C values of different components (root, stem and leaf) of *C. juncea* as cited in the literature (Abdul-Baki et al., 2001) and applied to the relative weight of these components measured here. The total respiratory output (R_d) was calculated by multiplying the average daily respiratory rates on a per second basis (determined by measuring CO_2 flux from the soil with the CIRAS-2 infrared gas analyzer) by the number of days, daytime hours, minutes and seconds during the experimental period. The total daytime respiratory output multiplied by the molecular weight of carbon (12 g/mol) yields the grams of carbon given off by daytime respiration during the experimental period. We assume that during the day stem and leaf respiration is negligible and therefore not measured. If however, stem or leaf respiration is substantive, our assumption can lead to an underestimation of R_d and an overestimation of recycling.

2.3. Calculation of gross photosynthesis and respiration by isotopic mass balance equations

We calculated gross photosynthesis and respiration by isotopic mass balance principles in the control plot under two scenarios, one without recycling and the other with recycling, and compare the results from these scenarios.

Biomass carbon formed in the cover crop stand ($BC = B\,\%C$) is the net effect of total gross photosynthesis (P) and total gross respiration (R) during the growth period:

$$BC = P - R. \quad (9)$$

By isotopic mass balance principles the following equation is derived:

$$BC\,\delta_B = P(\bar{\delta}_{AD} - \bar{\Delta}_{C-R}) - R\,\bar{\delta}_R, \quad (10)$$

This equation in principle is similar to those proposed by several authors (Yakir and Wang, 1996; Bowling et al., 2001, 2003; Ogée et al., 2003), but differs from their equations in that the values are integrated over the period of biomass formation.

Implicit in the above equation is the assumption, as the above authors have made, that there is no refixation of respired CO_2. In this case, the isotopic composition of the photosynthetically absorbed CO_2 is only a function of the isotopic composition of atmospheric CO_2 and the average value of canopy level discrimination. The average value of canopy level discrimination under the scenario that recycling does not occur, denoted here as $\bar{\Delta}_{C-R}$, is calculated by:

$$\bar{\Delta}_{C-R} = \bar{\delta}_{AD} - \bar{\delta}_{Leaf}. \quad (11)$$

The sole source for CO_2 fixation is CO_2 from above the canopy ($\bar{\delta}_{AD}$). Since this is a fast growing agricultural crop under constant irrigation conditions we can assume that the leaf carbon isotope ratios are directly related to discrimination as it has been observed for several agricultural crops (Richards and Condon, 1993). We simultaneously solved Eqs. (9) and (10) giving the solution to gross photosynthesis and respiration over the growth period under the first scenario where no recycling is occurring in the vegetation stand.

It is likely, however, that there are many vegetation canopies (including the one tested here) where the refixation of respired CO_2 occurs to a considerable amount (Sternberg et al., 1997). If this is the case, Eq. (9) must be modified to:

$$BC = (P_R + P_A) - (R_C + R_P) = P_A - R_C, \quad (12)$$

where the total gross photosynthesis ($P_R + P_A$) is composed of two components: the photosynthetic fixation of respired CO_2 (P_R) and photosynthetic fixation of atmospheric CO_2 (P_A). Likewise, the total gross respiration flux ($R_C + R_P$) is composed of two components: the total respiratory flux of CO_2 lost through the canopy (R_C, i.e. respiratory CO_2 that is not fixed by photosynthesis and is lost during the growth period) and the total respiratory flux that is fixed by photosynthesis (R_P). By definition: $P_R = R_P$ and these terms cancel out. Therefore, BC is the difference between the total atmospheric CO_2 taken up by photosynthesis and the total flux of respired CO_2 leaving the canopy (Eq. (12)).

The isotopic identity of respired CO_2 leaving the canopy of the vegetation during the day ($\bar{\delta}_{CD}$), however, will not be the same as that at its source in the case where recycling is high. Photosynthetic refixation of respired CO_2 and its associated discrimination will cause the remaining respired CO_2 to become isotopically enriched. This enrichment can be approximated by solving the following mass balance equation for $\bar{\delta}_{CD}$:

$$\delta_R = \bar{\Phi}_R(\bar{\delta}_R - \bar{\Delta}_{C+R}) + (1 - \bar{\Phi}_R)\bar{\delta}_{CD}. \quad (13)$$

This equation simply states that the isotopic composition of respired CO_2 (δ_R) can be expressed as the relative contribution of two proportions. The first is the proportion of respired CO_2 that is fixed by photosynthesis ($\bar{\Phi}_R$) with its respective isotopic ratio ($\bar{\delta}_R - \bar{\Delta}_{C+R}$). The second is the proportion of respired CO_2 that is not fixed by photosynthesis ($1 - \bar{\Phi}_R$) and its respective isotopic ratio ($\bar{\delta}_{CD}$), the value for which we solve. This equation shows that the greater the fixation of respired CO_2 the more isotopically enriched the remaining fraction of the respired CO_2 will be. In order to calculate canopy discrimination ($\bar{\Delta}_{C+R}$)under this scenario it is necessary to account for the presence of respired CO_2 in the canopy as a possible CO_2 source. Canopy discrimination in this case is calculated by solving the following equation for ($\bar{\Delta}_{C+R}$):

$$\delta_{Leaf} = [\bar{\Phi}_P(\bar{\delta}_R - \bar{\Delta}_{C+R})] + [(1 - \bar{\Phi}_P) \times (\bar{\delta}_{AD} - \bar{\Delta}_{C+R})]. \quad (14)$$

This mass balance equation states that the $\delta^{13}C$ values of leaf tissue (δ_{Leaf}) is the result of the contribution of two components. The proportion of material generated from the fixation of respired CO_2 ($\bar{\Phi}_P$) with its respective $\delta^{13}C$ value ($\bar{\delta}_R - \bar{\Delta}_{C+R}$), and the proportion of plant material generated from the fixation of atmospheric CO_2 ($1 - \bar{\Phi}_P$) with its respective isotopic ratio ($\bar{\delta}_{AD} - \bar{\Delta}_{C+R}$).

The isotopic ratio of CO_2 leaving the canopy over a 24 h period ($\bar{\delta}_C$)is the weighted average of the $\delta^{13}C$ value of CO_2 leaving the canopy during the day

(Eq. (14)) and that of respired CO_2 leaving the canopy during the night ($\bar{\delta}_R$) and is given by the following equation:

$$\bar{\delta}_C = \frac{\bar{\delta}_R + (1 - \bar{\Phi}_R)\bar{\delta}_{CD}}{2 - \bar{\Phi}_R}. \quad (15)$$

The isotopic ratio of respiratory CO_2 leaving the canopy over a 24 h period must be weighed because the quantity of respiratory CO_2 leaving the canopy during the day, by its partial consumption during recycling, is less than that leaving at night. It is assumed here that the respiration rates measured during the day are the same as night and that ground respiration makes up the bulk of gross respiration.

Using mass balance principles and Eq. (12):

$$BC\,\delta_B = P_A(\bar{\delta}_{AD} - \bar{\Delta}_{C+R}) - R_C\,\bar{\delta}_C. \quad (16)$$

Solving Eqs. (12) and (16) simultaneously will not yield gross respiration and photosynthesis, but the total flux of respiratory CO_2 leaving the vegetation canopy and the photosynthetic flux of CO_2 from above the canopy during the growth period. Knowing $\bar{\Phi}_R$, however, one can easily calculate gross respiration with:

$$R = R_C\left(\frac{2}{2 - \bar{\Phi}_R}\right) \quad (17)$$

and gross photosynthesis (with Eq. (9)). We solved Eqs. (12) and (16) simultaneously for the total amount of respired CO_2 leaving the canopy and the total photosynthetic uptake of CO_2 from above the canopy. Eq. (17) was then used to determine the gross respiration followed by the solution for gross photosynthesis with Eq. (9). These results gave us the values of gross photosynthesis and respiration under the second scenario in which recycling is present.

3. Methods

3.1. Study site and species

The site is located in the University of Florida Tropical Research and Education Center (TREC) in Homestead, FL (80.50°W, 25.51°N). The soils in this area are shallow and composed of a gravelly loam (loamy skeletal, carbonatic, hyperthermic, Lithic Undorthents) with an average pH of 7.6, EC of 0.33 ds/m and 14 mg kg^{-1} organic carbon (Nobel et al., 1996).

Crotalaria juncea L. (*Fabaceae*), commonly called Sunn Hemp, a C_3 tropical herbaceous annual native of India and Pakistan, was chosen for this experiment. It is a highly productive nitrogen-fixing cover crop that forms a dense canopy about 2 m high within 90 days of sowing and has produced 560–1905 g/m^2 of dry biomass at TREC (Li et al., 2000).

3.2. Plot layout

The treatment and control plot each consisted of a 30 m × 30 m area that was sowed with *C. juncea* L. on 18 March 2003. The plots were located 750 m apart on opposite ends of the TREC facilities to prevent contamination of the control plot by label emitted in the treatment plot. Embedded in the center of the planted area was a 10 m × 10 m plot that received fumigation. The embedded design was intended to minimize the dilution of label gas by edge effects. A grid of polyethylene hoses within the embedded plots was laid out ~10 cm above the soil surface for distribution of carrier gas with and without enriched CO_2, depending on the plot (Fig. 1). The grid had 100 points, equally spaced 1 m apart, which allowed gas emission from the terminal end of a 3 mm outer diameter (o.d.) tubing. The length of tubing from the source to each emitter was kept constant to ensure homogeneous air pressure at each emission point (Fig. 1).

Timing and flow rate of the gas delivered through the emission grid was controlled by a system protected by a tent several meters outside of the growing area. The emission grid was connected by 6.3 mm (o.d.) polyethylene tubing to a flow meter (Aalborg Instruments and Controls, Inc. Orangeburg, NY, USA) that controlled the flow rate of gas from the source to the grid. The flow meter was powered by a 12 V battery that was continually recharged during daylight hours by a solar panel. The gas delivered to the treatment plot was a mixture of 115 l of 41 at.% $^{13}CO_2$ ($\delta^{13}C \sim 63{,}000‰$) with 3240 l of 99.9995% nitrogen as a carrier gas (Icon Services Inc., Summit, NJ, USA). This gas was passed through a hydrocarbon trap (VICI Mat/Sen T200-2, Valco Instruments Co. Inc., Valco International). In order to minimize loss of costly labeled gas, flow was halted by a light detector

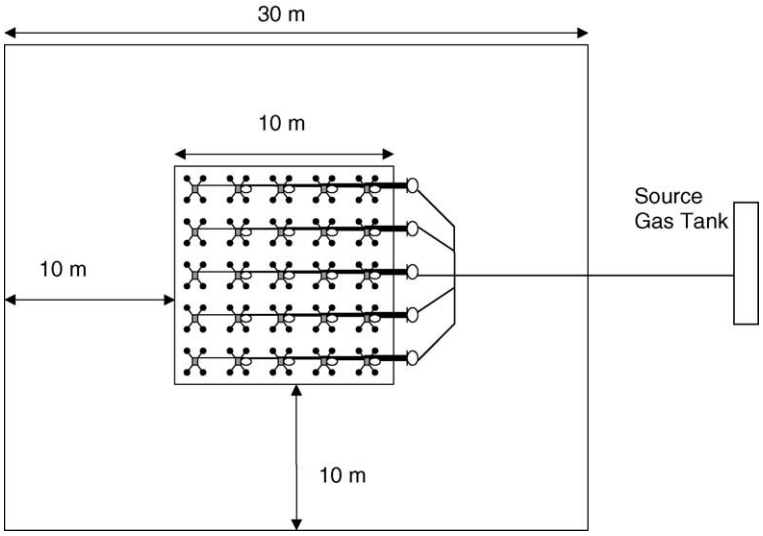

Fig. 1. Schematic diagram of the plot layout. Each plot consisted of a 30 × 30 area planted with *C. juncea*. Embedded within the center of each plot was a 10 m × 10 m area that received fumigation by means of a grid of polyethylene hoses that were connected to a source gas tank (medical grade nitrogen in the control plot and a mixture of 115 l of 41 at.% $^{13}CO_2$ with 3240 l of 99.9995% nitrogen as a carrier gas in the treatment plot). There were 100 equally spaced points of emission (●) within the fumigation area that delivered gas from the terminal end of a 3 mm (outer diameter) tubing. The length of tubing from the source gas was equal to ensure homogeneous air pressure at each emission point. The tubing was coiled (○) when its length exceeded that which was needed for a given cluster of emission points.

circuit that cut power to the flow meter during the night and resumed power at day break. Flow of labeled gas was allowed during the night only during ambient air sampling. This did not affect our experimental results because the aim of the experiment was to trace the amount of labeled respiratory CO_2 fixed during photosynthesis. Gas delivered to the control plot, which was also halted during night, was composed only of medical grade nitrogen gas.

3.3. Calculation of flow rate

Fumigation of plots began after the fourth week of growth when mean plant height ranged from 43.9 to 52.2 cm in each plot. Therefore, some biomass was formed before labeling which could lead to errors in our estimation of recycling. However, because the increase in girth and lignification of stems occurred at a later stage (∼1 m height), we propose that the bulk of the biomass was formed during labeling. Fumigation flow rate was calculated so that the labeled CO_2 flowed at a rate of 0.2% of the midday soil respiratory flux. Because treatment gas was so enriched in ^{13}C it was possible to alter the $\delta^{13}C$ value of respired CO_2 without significantly increasing the CO_2 flux from the ground level. Soil respiration rates were measured weekly and the flow rates adjusted accordingly.

The respiration rates used to calculate fumigation gas flow rates were the means of measurements taken at ten respiration sampling stations per embedded plot within both the control and treatment areas. Stations consisted of a polyvinyl chloride (PVC) pipe with an outside diameter of 12 cm imbedded 2.5 cm below the soil surface and protruding 10 cm above ground. During measurements, the soil chamber (SRC, PP Systems, UK) was tightly fitted into the above ground portion of the sampling station so that the seal prevented leakage. The installation of these PVC rings prevented the soil chamber from piercing the soil during measurement, a process that might cause injury to roots and consequential increase in CO_2 production that would confound measurement of CO_2 from soil respiration. Soil respiration was measured with a CIRAS-2 portable infrared gas analyzer (PP Systems, UK).

During week 10, 12, 13, and 14 leaf gas exchange was measured with a leaf cuvette connected to a portable gas analyzer (CIRAS-2, PP Systems, UK) at a light intensity of 1000 μmol m^{-2} s^{-1} for 12 leaves in each of the plots. Chamber relative flow rate was set at 200 ml min^{-1}, and CO_2 concentration was 375 ppm.

Internal CO_2 concentration of leaves (C_i) was compared between plots to monitor effects of leaf physiology on the photosynthetic isotopic carbon discrimination.

3.4. Collection of gas samples

Before sowing of seeds, a 5.2 m aluminum radio tower was constructed in the center of each plot. Six 1.27 cm OD metal/plastic composite tubes with an inner layer of aluminum surrounded by a high-density polyethylene jacket (Dekoron type 1300, Flexicon, Baton Rouge, LA) were laid from the collection tent outside of the 30 m × 30 m plot to the tower. Each tube was then fixed at each of the following heights 0.5, 1.0, 1.5, 2.0, 3.0 and 5.0 m. Once affixed, the terminal ends were turned towards the ground to prevent rainwater entry and covered by a screen cloth to prevent insect entry and nest-building.

Ambient air samples were collected in ~500 cm^3 pre-evacuated flasks with a manifold described by (Moreira et al., 1997). Air was pumped through the manifold for at least one minute before the collection flask was opened. Once the air sample entered the flask CO_2 concentration was measured (Li-6251, LI-COR Inc., Lincoln, Nebraska). Finally, the flask was sealed, and the air sample taken to the UM stable isotope lab for analysis. Ambient air samples were collected 8, 10, 12, and 14 weeks after sowing in the control plot and 12 and 14 weeks after sowing in the treatment plot during the night, while samples were collected three times in both plots during the day (10, 12, and 14 weeks after sowing) 2–6 h after sundown. Flow of labeled gas remained on at night during night sampling in the treatment plots. Three replicates were collected at each height at each sampling time (n = 18/plot). Additionally, during week 14, five gas samples were collected at night from the control plot into ~250 cm^3 flasks using methods described by Sternberg et al. (1997). These samples were collected in a chamber placed on top of the soil surface and the CO_2 concentration allowed to increase to about 600 ppm. These samples therefore represent a mixture of air and respired CO_2 having a greater concentration of respired CO_2 when compared with ambient air samples.

3.5. Biomass harvest

Plant biomass was harvested after the 14th week of growth. All biomass was collected from ten 0.5 m^2 sampling areas, spaced 2 m apart and aligned along a transect that extended from the center of the outer edge of the 30 × 30 plot through the area of fumigation. Five areas each were sampled from the fumigated and non-fumigated sections of the treatment and control plots. Root, stem and leaf tissues were kept separate, dried at 70 °C until they reached a constant weight, weighed and then ground (Intermediate Thomas Wiley Cutting Mill with size 20 mesh, 850 μm aperture).

3.6. Stable isotope analysis of air, soil respiration and biomass samples

Flasks containing air samples were fitted into a vacuum system in which CO_2 was separated from air and water by cryogenic distillation. The CO_2 was then sealed within a pre-evacuated glass ampoule with pure copper and heated to 450 °C for 2 h, to eliminate any nitrous oxides which interfere with the measurement of the carbon isotope ratio (Craig and Keeling, 1963).

Carbon dioxide was extracted and purified from approximately 5 mg of biomass tissue based on the methods of Buchanan and Corcoran (1959).

Carbon isotope ratios of the purified CO_2 were measured by mass spectrometry (VG Prism, Micromass, Middlebury, UK) and expressed as:

$$\delta^{13}\mathrm{C}\,(‰) = \left[\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}} - 1\right] \times 1000, \tag{18}$$

where R_{sample} and R_{standard} are the $^{13}C/^{12}C$ ratios of sample and standard which is the *Belemnita americana* fossil carbonate from the Pee Dee geological formation in South Carolina (VPDB), respectively. The precision of analysis is ±0.1‰ (1σ).

4. Results and discussion

4.1. Respiratory based recycling index calculated from the modified Keeling plot

Night-time measurements of $\delta^{13}C$ values of ambient CO_2 were highly correlated with the inverse of the ambient CO_2 concentration in the control plot (r = 0.97, $p < 0.01$, geometric mean regression) after eliminating three outlier points, with an intercept of −26.16‰ (Fig. 2). The $\delta^{13}C$ value of respiration in the

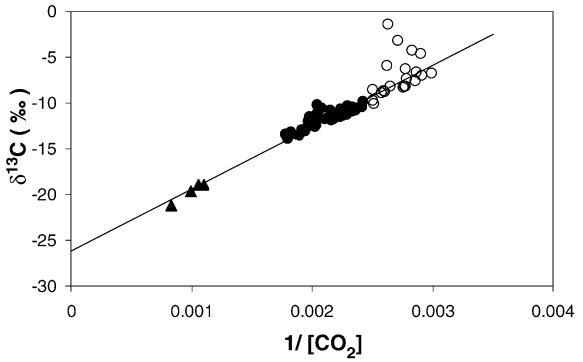

Fig. 2. Keeling plot was calculated from air (●) ($n = 70$) and soil respiration samples (▲) ($n = 4$) that were collected at night in the control plot. The geometric mean regression is significant ($p \leq 0.01$, $y = 6767.15x - 26.16$, $n = 74$, $r = 0.97$). Air samples that were collected during the day from the understory (○) ($n = 21$) of the control plot were used to calculate recycling under steady-state assumptions.

control plot calculated with Eq. (2), however, was lower averaging −28.56‰. We will use this latter value as representative of the isotopic ratios of respired CO_2 to draw the Keeling plot. The low organic content of the soils at this site leads us to the conclusion that $\delta^{13}C$ value of respired CO_2 represents the value from that of plant root respiration (so called autotrophic respiration). Interestingly the estimated $\delta^{13}C$ value of respired CO_2 (−28.56‰) is very similar to that of root material collected at this plot (averaging −28.7‰), supporting the conclusion that there is no fractionation during respiration (Lin and Ehleringer, 1997). Daytime isotopic and concentration measurements of ambient CO_2 in the control plot deviate significantly from the straight line Keeling equation derived from night-time samples (Fig. 2). This deviation can be used to calculate Φ_R according to Eq. (3). Calculation of recycling for several of the daytime measurements (9 out of 21 measurements) gave unrealistic results either greater than 1 or negative. Most of the points, which gave unrealistic values, were above the Keeling line (Fig. 2).

These out of range point measurements may have been caused by this system not being in steady state, a violation of one basic assumption of the model (Sternberg, 1989). Alternatively, it is possible that the outlier points were sampled at a time when leaf level discrimination differed considerably from the average of 18.96‰ estimated here. Calculating Φ_R only for the points within the range of possible values yielded the average recycling value of 0.41 ± 0.03 S.E.M. ($n = 12$). This theoretical method of calculating recycling, however, is complicated by having some of the points used in the calculation of recycling indistinguishable from those within the confidence limit of the Keeling line. Therefore, it is impossible to determine whether the deviation of a data point from the Keeling line is experimental error or strongly affected by the recycling process.

4.2. Recycling calculated from differences in $\delta^{13}C$ values of biomass in treatment and control plots

The $\delta^{13}C$ values of all plant parts, leaf, stem and roots of treatment plants were significantly greater

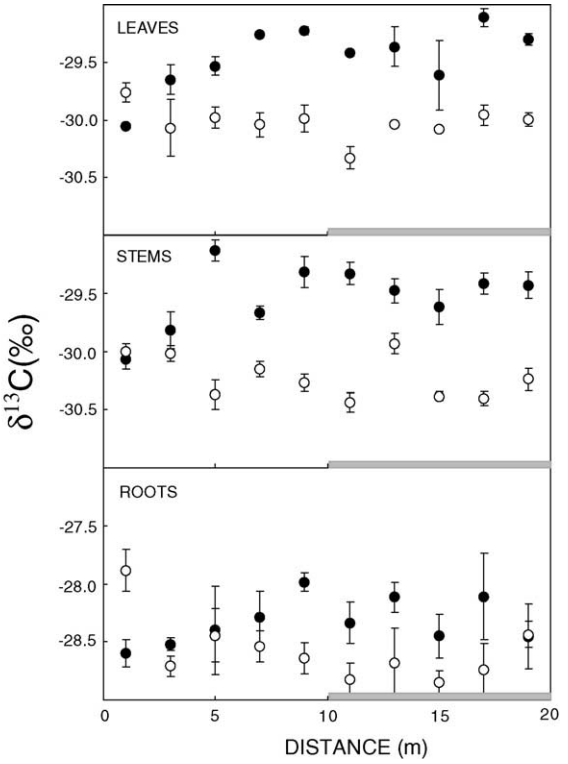

Fig. 3. Mean (±S.E.M.) carbon isotope values of root, stem and leaf biomass that was harvested 14 weeks after seeds were sown in the treatment (●) and control (○) plots. Stippled bar indicates the embedded area that received either unlabeled or labeled fumigation within the control and treatment plots, respectively. Samples of all three tissue types from within the fumigated area of the treatment plot are more enriched in $\delta^{13}C$ and significantly different than those from the control plot (t-test, $p < 0.05$).

than those of the control plot (Fig. 3, Table 1). Differences were most pronounced in leaf and stem samples. The smaller differences in $\delta^{13}C$ values between roots of plants in the treatment and control plot could be the result of the bulk of roots being formed previous to the CO_2 label application. Isotopic differences between treatment and control plots extended to the areas of the 30 m × 30 m plot outside the fumigation area indicating a considerable advective loss of label. Only small differences were observed at the very edge of the 30 m × 30 m plot caused by complete dilution of label at the very edge of the plots (Fig. 3). Leaf internal CO_2 concentration, a major factor regulating the isotopic discrimination by C_3 plants (Farquhar et al., 1982), was not significantly different between treatment (243.36 ± 3.62 ppm) and control (241.53 ± 2.80 ppm) plots (*t*-test, $p = 0.69$, $n = 36$). The differences in biomass $\delta^{13}C$ values between treatment and control plots cannot be ascribed to differences in discrimination brought about by differences in gas exchange properties between plants in treatment and control plots.

Further evidence to this conclusion is the observation that there were minimal isotopic ratio differences between treatment and control plots at the very edge of the plots where the dilution of the label is complete. These differences were therefore due to the differences in the $\delta^{13}C$ values of respired CO_2 in the control plot and labeled respired CO_2 in the treatment plot, indicating that recycling occurs in these two plots. Although the regression coefficient for the Keeling equation in the control plot was high ($r = 0.97$), the regression coefficient for the treatment plot was low adding uncertainty to the value of respired CO_2 derived by the Keeling plot ($r = 0.48$, $p < 0.05$). As in the control, we calculated isotopic composition of respired CO_2 in the treatment plot with equation 2, giving it a value of −23.89 ± 3.7‰. Solving Eqs. (5) and (6) simultaneously we derive a photosynthesis based recycling index ($\bar{\Phi}_P$) of 0.183 (Table 1). In other words, 18.3% of the biomass produced in the two vegetation stands grown here is from respired CO_2. Solving Eq. (8) for Φ_R indicates that 49% and 48% of the daytime respired CO_2 from ground level is recycled by photosynthesis in control and treatment plots, respectively (Table 1). Approximately only 51% of the daytime respired CO_2 was released to the atmosphere above the canopy during the day. This recycling value was similar to the average recycling index based on the modified Keeling equation (0.49

Table 1
Definitions and values of some critical parameters used in the calculation of respiratory based recycling by two methods: modified Keeling equation and a mass balance approach

Symbol	Definition	Values
Measurement of Φ_R with modified Keeling equation		
$\bar{\delta}_R$	Average $\delta^{13}C$ value of respired CO_2 in control plot calculated with Eq. (2) (‰): $N = 74$	−28.56 ± 3
$\bar{\delta}_{AD}$	Average $\delta^{13}C$ value of atmospheric CO_2 ~5 m above the ground during the day (‰)	−8.12
$\bar{\Delta}_L$	Average leaf level photosynthetic fractionation calculated from Eq. (4) (‰)	18.96
Φ_R	Average respiratory based recycling index calculated using the modified Keeling Eq. (3): $N = 12$	0.41 ± 0.03

	Biomass produced (g m^{-2})	Control	Treatment
Measurement of Φ_R with isotope ratios of biomass and mass balance equations			
B	Leaf	244	162
	Root	154	220
	Stem	1246	927
	Total	1644	1309
δ_B, δ'_B	Average $\delta^{13}C$ value of biomass (‰)	−30.1	−29.22
$\bar{\delta}_R, \bar{\delta}'_R$	Average $\delta^{13}C$ value of respired CO_2 (‰)	−28.56	−23.89
$\bar{\Phi}_P$	Photosynthetically based recycling index calculated with Eq. (7)	0.183	
$\bar{\Phi}_P B$ %C	Total amount of carbon from respiration which is fixed into biomass (g m^{-2})	134.4	106.5
R_d	Total daytime molar output of respired CO_2 during the experiment (mol m^{-2})	22.83	18.30
R_d 12	Total amount of carbon given off by respiration during the day for 63 days (g m^{-2})	274.0	219.5
$\bar{\Phi}_R$	Respiratory based recycling index calculated with Eq. (8)	0.49	0.48

versus 0.41). The discrepancy between the biomass based recycling index and that calculated by the modified Keeling equation could be due to two reasons in addition to the problems with the theoretical method mentioned in the previous section. First, the recycling index based on the Keeling plot represents recycling for data collected for 3 days only, whereas the recycling index based on data from biomass represents recycling over the whole period of biomass generation. Second, when calculating the respiratory based recycling index with $\delta^{13}C$ values of biomass, we assume that the only source of respiration is that which was measured; ground respiration. Stem and night-time leaf respiration in this cover crop could be substantial and if it represents, as a typical example, 20% of the measured ground respiration, then the biomass based Φ_R would lower to a value of 0.41, similar to that measured with a modified Keeling equation.

4.3. Calculations of gross respiration and photosynthesis under two scenarios: recycling and no recycling

Eqs. (9) and (10) were solved simultaneously for the control plot under the scenario where it is assumed that no refixation of respired CO_2 is occurring. In this case all of the photosynthetic CO_2 uptake is directly from the air above the canopy and has that particular isotopic signature (-8.12 ± 0.37‰, Table 1). Likewise the CO_2 released from respiration through the canopy will have the same isotopic signature as that measured using the rearranged Keeling equation (Eq. (2)) from night-time measurements (-28.56 ± 3.0‰, Table 2). Gross photosynthesis and respiration under this scenario when converted to the familiar units of $\mu mol\ m^{-2}\ s^{-1}$ were calculated as 22.75 and 0.105 $\mu mol\ m^{-2}\ s^{-1}$, respectively (Table 2). These values are unrealistically low and much lower than previously reported values for agricultural crop stands (Yakir and Wang, 1996). Further, the average gross respiration rate calculated here (0.105 $\mu mol\ m^{-2}\ s^{-1}$) is much lower than the average soil respiration measured with the CIRA-2 infrared gas analyzer (8.39 $\mu mol\ m^{-2}\ s^{-1}$).

If recycling is taken into account, however, the $\delta^{13}C$ value of respired CO_2 leaving the canopy during the day according to Eq. (13) is -11.01‰. However the average $\delta^{13}C$ value of respired CO_2 leaving the canopy during a 24-h period is -22.63‰ (Eq. (15), Table 2). The average photosynthetic rate of CO_2 being fixed from sources above the canopy is calculated to be 45.43 $\mu mol\ m^{-2}\ s^{-1}$ and the rate of respired CO_2 leaving the canopy averages 11.45 $\mu mol\ m^{-2}\ s^{-1}$. However, in the case where recycling occurs, these values do not represent gross photosynthesis or respiration which must be solved using Eq. (17) for gross respiration and Eq. (9) for gross photosynthesis. The values calculated under this scenario are 52.88 and 15.17 $\mu mol\ m^{-2}\ s^{-1}$ for gross photosynthesis and

Table 2
Definitions and values of variables used in the calculation of gross photosynthesis and respiration under two scenarios: recycling and no recycling

Symbol	Definition	Values	
BC	Biomass carbon formed in the cover crop stand in the control plot	$735.9 \pm 93.8\ g\ m^{-2}$	
		No recycling	Recycling
$\bar{\Delta}_{C-R}, \bar{\Delta}_{C+R}$	Canopy level photosynthetic discrimination—takes into account draw down of carbon dioxide concentration	21.96‰	18.23‰
δ_C	Isotopic value of CO_2 leaving the canopy of the vegetation during the day	−28.56‰	−11.01‰
$\bar{\delta}_C$	Isotopic value of CO_2 leaving the canopy of the vegetation during a 24-h period	−28.56‰	−22.63
R_C	Respiratory carbon (g) that is not fixed by photosynthesis and is lost during the growth period	6.86, 0.105 $\mu mol\ m^{-2}\ s^{-1}$	747.9, 11.45 $\mu mol\ m^{-2}\ s^{-1}$
P_A	Photosynthetic fixation of atmospheric carbon (g)	742.86, 22.75 $\mu mol\ m^{-2}\ s^{-1}$	1483.89, 45.43 $\mu mol\ m^{-2}\ s^{-1}$
R	Total gross respiration of carbon (g)	6.86, 0.105 $\mu mol\ m^{-2}\ s^{-1}$	990.92, 15.17 $\mu mol\ m^{-2}\ s^{-1}$
P	Total gross photosynthetic fixation of carbon (g)	742.86, 22.75 $\mu mol\ m^{-2}\ s^{-1}$	1726.92, 52.87 $\mu mol\ m^{-2}\ s^{-1}$

respiration, respectively. These values are similar to previous measurements in dense agricultural crops and forests (Yakir and Wang, 1996; Bowling et al., 2001).

5. Conclusions

Respiratory based recycling was high regardless of which method was used in the calculation: 0.41 based on the modified Keeling equation and 0.49 based on the fumigation experiment. Both methods gave similar recycling indices. However, the theoretical method suffers from having points that yield nonsensical values of recycling or that are indistinguishable from those caused by measurement errors. The differences between these two methods may have been caused by the following major reasons. First, the modified Keeling plot method not only has the above-cited problems, but was also used for measurements only during 3 days of the growth period, whereas the calculation by the fumigation method integrates recycling throughout the period of growth. Second, respiration could have been underestimated which would lead to an increase in Φ_R as calculated by the biomass method. These high recycling values may be more frequently found in densely planted cover crops as was shown here. High recycling values would also be encountered in dense canopies such as in tropical forests (Sternberg et al., 1997). In more open vegetation canopies such as savannas and open forests, however, recycling is probably not important. When recycling is high and its impact on the isotopic composition of respiratory CO_2 is ignored, inaccurate levels of gross photosynthesis and respiration will be calculated by the isotopic mass balance approach. In this particular case, when recycling is not taken into account, gross respiration is underestimated by an order of magnitude. Modeled predictions on the effect of climate change in NEE based in these inaccurate estimates of gross respiration and photosynthesis will be unreliable.

Acknowledgements

This research was supported by NIGEC grant 02UOM01. Many of the ideas presented here were developed during discussions with Dr. Steve Oberbauer (Department of Biology, Florida International University).

References

Abdul-Baki, A.A., Bryan, H.H., Zinati, G.M., Klassen, W., Codallo, M., Heckert, N., 2001. Biomass yield and flower production in Sunn Hemp: effect of cutting the main stem. J. Veg. Crop. Prod. 7, 83–104.

Baldocchi, D.D., Law, B.E., Anthoni, P.M., 2000. On measuring and modeling energy fluxes above the floor of a homogeneous and heterogeneous conifer forest. Agric. For. Meteorol. 102, 187–206.

Bowling, D.R., Tans, P.P., Monson, R.K., 2001. Partitioning net ecosystem carbon exchange with isotopic fluxes of CO_2. Global Change Biol. 7, 127–145.

Bowling, D.R., Pataki, D.E., Ehleringer, J.R., 2003. Critical evaluation of micrometeorological methods for measuring ecosystem-atmosphere isotopic exchange of CO_2. Agric. For. Meteorol. 116, 159–179.

Buchanan, D.L., Corcoran, E.J., 1959. Sealed tube combustion for the determination of ^{14}C and carbon isotopes. Anal. Chem. 31, 1635–1638.

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

Craig, H., Keeling, C.D., 1963. The effects of atmospheric NO_2 on the measured isotopic composition of atmospheric CO_2. Geochim. Cosmochim. Acta 27, 549–551.

Farquhar, G.D., O'Leary, M.H., Berry, J.A., 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Ann. Rev. Plant Physiol. Plant Mol. Biol. 9, 121–137.

Goulden, M.L., Munger, J.W., Fans, S.-M., Daube, B.C., Wofsy, S.C., 1996. Measurements of carbon sequestration by long term edy covariance: methods and a critical evaluation of accuracy. Global Change Biol. 2, 169–182.

Houghton, R.A., 1991. Tropical deforestation and atmospheric carbon dioxide. Climate Change 19, 99–118.

Keeling, C.D., 1961. The concentration and isotopic abundances of carbon dioxide in rural and marine air. Geochim. Cosmochim. Acta 24, 277–298.

Law, B.E., Ryan, M.G., Anthoni, P.M., 1999. Seasonal and annual respiration of a ponderosa pine ecosystem. Global Change Biol. 5, 169–182.

Li, Y., Herbert, B., Olczyk, T., 2000. Sunn-Hemp—a cover crop in Florida. SS-AGR-96 Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, pp. 1–2.

Lin, G., Ehleringer, J.R., 1997. Carbon isotopic fractionation does not occur during dark respiration in C_3 and C_4 plants. Plant Physiol. 114, 391–394.

Lloyd, J., Kruijt, B., Hollinger, D.Y., Grace, J., Francey, R.J., Wong, S.-C., Kelliher, F.M., Miranda, A.C., Farquhar, G.D., Gash,

J.H.C., Vygodskaya, N.N., Wright, I.R., Miranda, H.S., Schulze, E.-D., 1996. Vegetation effects on the isotopic composition of atmospheric CO_2 at local and regional scales: theoretical aspects and a comparison between rain forest in Amazonia and a Boreal forest in Siberia. Aust. J. Plant Physiol. 23, 371–399.

Lloyd, J., Kruijt, B., Hollinger, D.Y., Grace, J., Francey, R.J., Wong, S.C., Kelliher, F.M., Miranda, A.C., Farquhar, G.D., Gash, J.H.C., Vygodskaya, N.N., Wright, I.R., Miranda, H.S., Schulze, E.D., 1997. An alternative interpretation of the appropriateness and correct means for the evaluation of CO_2 recycling indices. Aust. J. Plant Physiol. 24, 399–405.

Moreira, M.Z., Sternberg, L.da S.L., Martinelli, L.A., Victoria, R., Bonates, L.C.M., Nepstad, D.C., 1997. Contribution of transpiration to forest ambient vapour based on isotopic measurements. Global Change Biol. 3, 439–450.

Nobel, C.V., Drew, R.R.W., Slabaugh, J.D., 1996. Soil Survey of Dade County area, Florida. US Department of Agriculture NRCS, Washington, DC.

Ogée, J., Peylin, P., Ciais, P., Bariac, T., Bruent, Y., Roche, C., Richard, P., Bardoux, G., Bonnefond, J.-M., 2003. Partitioning net ecosystem carbon exchange into net assimilation and respiration using $^{13}CO_2$ measurements: a cost-effective sampling strategy. Global Biogeochem. Cycles 17, 39 1–39 18.

Pataki, D.E., Ehleringer, J.R., Flanagan, L.B., Yakir, D., Bowling, D.R., Still, C.J., Buchmann, N., Kaplan, J.O., Berry, J.A., 2003. The application and interpretation of Keeling plots in terrestrial carbon cycle research. Global Biogeochem. Cycles 17, 1–14.

Raupach, M.R., Denmead, O.T., Dunin, F.X., 1992. Challenges in linking atmospheric CO_2 concentrations to fluxes at local and regional scales. Aust. J. Bot. 40, 697–716.

Richards, R.A., Condon, A.G., 1993. Challenges ahead in using carbon isotope discrimination in plant-breeding programs. In: Ehleringer, J.R., Hall, A.E., Farquhar, G.D. (Eds.), Stable Isotopes and Plant Carbon–Water Relations. Academic Press, San Diego, pp. 451–561.

Ryan, M.G., Hubbard, R.M., Pongracic, S., Rasion, R.J., McMurtrie, R.E., 1996. Foliage, fine-root, woody tissue and stand respiration in *Pinus radiata* in relation to nitrogen status. Tree Physiol. 16, 333–343.

Schimel, D.S., House, J.I., Hibbard, K.A., Bousquet, P., Ciais, P., Peylin, P., Braswell, B.H., Apps, M.J., Baker, D., Bondeau, A., Canadell, J., Churkina, G., Cramer, W., Denning, A.S., Field, C.B., Friedlingstein, P., Goodale, C., Heimann, M., Houghton, R.A., Melillo, J.M., Moore III, B., Murdiyarso, D., Noble, I., Pacala, S.W., Prentice, I.C., Raupach, M.R., Rayner, P.J., Scholes, R.J., Steffen, W.L., Wirth, C., 2001. Recent patterns and mechanisms of carbon exchange by terrestrial ecosystems. Nature 414, 169–172.

Schleser, G.H., Jayasekera, R., 1985. δ^{13}C-variations of leaves in forests as an indication of reassimilated CO_2 from the soil. Oecologia 65, 536–542.

Sternberg, L., 1989. A model to estimate carbon dioxide recycling in forest using $^{13}C/^{12}C$ ratios and concentrations of ambient carbon dioxide. Agric. For. Meteorol. 48, 163–173.

Sternberg, L., 1997. Interpretation of recycling indexes. In: Kruijt, L.B., Hollinger, D.Y., Grace, J., Francey, R.J., Wong, S.-C., Kelliher, F.M., Miranda, A.C., Farquhar, G.D., Gash, J.H.C., Vygodskaya, N.N., Wright, I.R., Miranda, H.S., Schulze, E.-D. (Eds.), A comment on 'Vegetation Effects on the Isotopic Composition of Atmospheric CO2 at Local and Regional Scales: Theoretical Aspects and a Comparison Between Rain Forest in Amazonia and a Boreal Forest in Siberia'. Aust. J. Plant Physiol. 23, 371–499 (1996); Aust. J. Plant Physiol. 24, 395–398.

Sternberg, L., Moreira, M.Z., Martinelli, L.A., Victoria, R.L., Barbosa, E.M., Bonates, L.C.M., Nepstad, D.C., 1997. Carbon dioxide recycling in two Amazonian tropical forests. Agric. For. Meteorol. 88, 259–268.

Sternberg, L.da S.L., DeAngelis, D.L., 2002. Carbon isotope composition of ambient CO_2 and recycling: a matrix simulation model. Ecol. Model. 154, 179–192.

Valentini, R., Matteucci, G., Dolman, A.J., Schulze, E.-D., Rebmann, C., Moors, E.J., Grainier, A., Gross, P., Jensen, N.O., Pllegaard, K., Lindroth, A., Grelle, A., Bernhofer, C., Grünwald, T., Aubinet, M.C.R., Kowalski, A.S., Vesala, T., Rannlk, Ü., Berblgier, P., Loustau, D., Guomundsson, J., Thorgeirsson, H., Ibrom, A., Morgenstern, K., Clement, R., Moncrieff, J., Montagnani, L., Minerbi, S., Jarvis, P.G., 2000. Respiration as the main determinant of carbon balance in European forests. Nature 404, 861–865.

Yakir, D., Sternberg, L.da S.L., 2000. The use of stable isotopes to study ecosystem gas exchange. Oecologia 123, 297–311.

Yakir, D., Wang, X.-F., 1996. Fluxes of CO_2 and water between terrestrial vegetation and the atmosphere estimated from isotope measurements. Nature 380, 515–517.