

Partitioning NEE for absolute C input into various ecosystem pools by combining results from eddy-covariance, atmospheric flux partitioning and ^{13}C pulse labeling

M. Riederer · J. Pausch · Y. Kuzyakov · T. Foken

Received: 17 March 2014 / Accepted: 22 December 2014 / Published online: 9 January 2015
© Springer International Publishing Switzerland 2015

Abstract

Background and aims The complexity of ecosystem processes, especially under continuously changing environmental conditions, requires high-resolution insight into ecosystem carbon (C) fluxes. It is essential to gain not only information about relative C balance and fluxes (common for partitioning studies), but also to obtain these in absolute mass units.

Methods To evaluate absolute fluxes in belowground C pools, the results of 21-day eddy-covariance and stable isotope labeling experiment in summer 2010, were combined. Eddy-covariance based net ecosystem exchange was measured on extensively managed grassland and separated into underlying assimilation and ecosystem

respiration through the use of a C flux partitioning model. Resultant CO_2 assimilation served as absolute C input into the ecosystem and was further partitioned by applying the relative C distribution in subsidiary pools, gained by ^{13}C pulse labeling and tracing.

Results The results from eddy-covariance measurements showed that the extensively managed grassland was a significant net C sink of $-91 \text{ g C m}^{-2} \text{ a}^{-1}$ in 2010.

The mean daily assimilation of $-7.1 \text{ g C m}^{-2} \text{ d}^{-1}$ was partitioned into fluxes of 2.5, 0.8, 0.5, 2.3 and $1.0 \text{ g C m}^{-2} \text{ d}^{-1}$ into shoots, roots, soil, shoot respiration and CO_2 efflux from soil, respectively.

Conclusions We conclude that the combination of EC measurements with isotope labeling techniques allowed determining the absolute C input into several ecosystem pools. Hence, the study demonstrates an approach to expand atmospheric flux measurements and to gain insight into the importance of individual ecosystem pools for soil C cycling.

Responsible Editor: Zucong Cai.

M. Riederer · T. Foken
Department of Micrometeorology, University of Bayreuth,
95440 Bayreuth, Germany

J. Pausch · Y. Kuzyakov
Department of Soil Science of Temperate Ecosystems,
University of Göttingen,
37077 Göttingen, Germany

T. Foken (✉)
Member of Bayreuth Center of Ecology and Environmental
Research (BayCEER), University of Bayreuth,
95440 Bayreuth, Germany
e-mail: thomas.foken@uni-bayreuth.de

M. Riederer (✉)
Regensburg Center of Energy and Resources, Regensburg
University of Applied Sciences,
93049 Regensburg, Germany
e-mail: riederer.michael@googlemail.com

Keywords Stable isotope pulse labeling · Net ecosystem exchange · Carbon flux · Extensively managed grassland

Introduction

Currently, two dominant approaches in ecosystem sciences are used to gain access to the carbon (C) cycle of terrestrial ecosystems. Micrometeorological methods like the eddy-covariance (EC) technique provide a top view from the atmosphere (Aubinet et al. 2000, 2012;

Baldocchi 2003; Baldocchi et al. 2001; Moncrieff et al. 1997), whereas leading isotopic methods used nowadays in agricultural and soil science allow a more interior view of the ecosystem (Kuzyakov and Domanski 2000; Yakir and Sternberg 2000). Both are occasionally combined with chamber methods to facilitate and expand investigation of CO₂ fluxes (Goulden et al. 1996; Davidson et al. 2002; Dore et al. 2003; Subke and Tenhunen 2004; Rochette and Hutchinson 2005). While EC methods have the advantage of barely disturbing ecosystem processes during the experiment, isotopic methods are mostly destructive due to the necessity of taking plant and soil samples. Another difference is that isotopic labeling approaches are largely point measurements, while EC integrates the signal throughout a large flux-footprint (Vesala et al. 2008).

EC is generally the favored technique on grasslands for measuring the C balance in terms of the net ecosystem carbon exchange (NEE), i.e., the proportion of C released and taken up by the ecosystem (Wohlfahrt et al. 2012). To evaluate underlying processes and responses of the ecosystem to environmental change, NEE has to be separated into its components: ecosystem respiration (R_{ECO}) and gross primary production (GPP), by flux partitioning models (FPM; Falge et al. 2002; Stoy et al. 2006; Desai et al. 2008; Lasslop et al. 2010; Reichstein et al. 2012). These are also used to gap-fill missing or rejected data (Stoy et al. 2006; Ruppert et al. 2006; Desai et al. 2008; Papale 2012; Falge et al. 2001; Moffat et al. 2007). By determining temporal variations and the absolute amount of assimilated and released C for a certain period, the atmospheric approach reaches its limits.

Further partitioning of total CO₂ efflux or C input (GPP) into various ecosystem pools is not possible based on EC, but can be achieved using isotopic techniques (Buchmann 2000, 2002; Kuzyakov 2006). Thereby, natural continuous (C3 plants grow after C4 plants or vice versa), artificial continuous and artificial pulse labeling approaches have to be differentiated. Advantages and disadvantages of the different labeling approaches were discussed in several publications (Whipps 1990; Nguyen 2009; Werth and Kuzyakov 2008). Pulse labeling provides the relative distribution of recently assimilated C into various above and below ground pools.

EC delivers the absolute C fluxes above the ecosystem. Combining the results of EC with that of ¹³CO₂

pulse labeling allows tracing the absolute input of C into various ecosystem pools.

Previous discussions in the literature about combining stable isotope methods with eddy-covariance technique were aimed at, for example, acquiring natural atmospheric iso-fluxes (Yakir and Sternberg 2000; Bowling et al. 2001; Wichura 2009) or, in the case of pulse labeling, evaluating and comparing the C cycle of various ecosystems (Gavrichkova 2009).

Today, European grasslands are predominantly considered as C sinks but there are uncertainties: the IPCC did not agree with this opinion and ascribed a potential role of either source or sink to grassland ecosystems (IPCC 2007). Janssens (2003) found a certain sink capacity but with an uncertainty that was larger than the sink itself. Also Ciais et al. (2010) could not sufficiently prove the detected sink capacity. Future climate change will even increase this uncertainty by affecting C cycling in temperate grasslands due to increasing temperatures (Luo 2007), varying precipitation amounts and patterns (Knapp 2002; Chou et al. 2008), heat waves and droughts (Ciais et al. 2005; Joos et al. 2010), and rising atmospheric CO₂ concentrations (Luo et al. 2006).

The present study was conducted at an extensively managed grassland site in Central Europe during the main vegetation period 2010. Besides addressing the question whether grassland ecosystems function as C sink or source, the main aim of the current experiment was to determine the absolute C input into various ecosystem pools.

For these reasons eddy-covariance measurements and a ¹³CO₂ pulse labeling experiment were conducted

To our knowledge, this is the first study combining results of EC measurements and of a CO₂ pulse labeling experiment to determine the absolute amounts of C transferred to various pools of a grassland ecosystem in Central Europe.

Methods

Study area

The experiment was conducted during summer 2010 from June 16th (DOY 167) to July 6th (DOY 187) on a submontane grassland site at the edge of the low mountain range “Fichtelgebirge”, 624 m a.s.l. (50°05' 25"N, 11°51'25"E) in northeast Bavaria, Germany. For

the last 10 years the experimental site was used as extensively managed grassland without fertilization or grazing, but with sporadic mowing once or twice a year. The soil type is gleysol (IUSS Working Group WRB 2007), with a thickness of at least 70 cm. The average annual temperature and precipitation are 5.8 °C and 1066 mm, respectively (Foken 2003). The “Großer Waldstein” (877 m a.s.l.) lies north of the study site and the “Schneeberg” (1051 m a.s.l.) is to the south. These two mountains generate a channeled wind field on the site with East and above all West as dominating wind directions (prevailing wind direction 263°). The plant community can be described as *Molinio–Arrhenatheretea* R. Tx. 1937 – economic grassland. With 48 species, the biodiversity is quite high. The most dominant species are *Alchemilla monticola*, *Juncus filiformis*, *Polygonum bistorta*, *Ranunculus acris* and *Trifolium repens*. These species were considered when to decide the exact location of the labeling plots to gain best possible comparability with the whole ecosystem. Except for single larger individuals, the canopy height was about 0.4 m at the date of labeling.

Micrometeorological determination of absolute C input

Experiment setup

An automated weather station provided 10 min averages of a range of climate data to evaluate short term effects, but also to provide the input parameters for the partitioning of the NEE into its source and sink components. The most important collected parameters were up- and down welling short- and long wave radiation, air and soil temperature, humidity and soil moisture and precipitation. High frequency (20 Hz, 2.5 m above ground) data were collected to determine turbulent fluxes, such as NEE by eddy-covariance. Water vapor and CO₂ concentration were measured by an open-path gas analyzer (LI-7500, LI-COR Biosciences, Lincoln, Nebraska USA) and wind vector and sonic temperature (TS) by a 3D sonic anemometer (CSAT3, Campbell Scientific, Inc., Logan, Utah USA). CSAT3 and LI-7500 were pointed in a northerly direction, normal to the prevailing wind direction of 263°. Thus, disturbance of the flux by the instruments was minimized (Li et al. 2013). Tower shading could be avoided completely due to the channeled wind regime. Data were stored on a data logger (CR3000, Campbell Scientific, Inc., Logan,

Utah USA) and collected daily by a computer system as a backup.

Data acquisition and analysis

The raw data for the turbulent CO₂ fluxes were post processed and quality controlled based on micrometeorological standards, applying the software package TK2 developed at the University of Bayreuth (Mauder and Foken 2004). This still evolving software (TK3 is now available; Mauder and Foken 2011) includes all necessary data correction and data quality tools (Foken et al. 2012), was proved in comparison with six other commonly used software packages (Mauder et al. 2008) and successfully applied in numerous major field campaigns (Mauder et al. 2006, 2007; Eigenmann et al. 2009). The included quality flagging system evaluated stationarity and turbulence during the averaging interval of 30 min and marked the resulting flux data with quality flags from 1 (very good quality) to 9 (very low quality; Foken and Wichura 1996; Foken et al. 2004). The flux data were then filtered according to these flags and only data with quality 3 or better were used during the whole experiment. In addition to that, footprint analysis was performed (Göckede et al. 2004, 2006). It could be assured that the signal measured by EC originated exclusively from the target land use type grassland (Rannik et al. 2012). Due to the channeled wind regime, two clubbed footprints evolved in western and eastern directions. Thus, disturbances of the turbulent fluxes measured by EC could be avoided by installing the other experimental devices directly adjacent to the EC mast but perpendicular to the main wind direction.

NEE flux partitioning

In order to finally gain absolute C input into the ecosystem from the NEE data, two tasks were performed: Due to rejection of outliers and low quality data, small gaps occurred within the 30 min NEE time series that had to be filled and the NEE had to be partitioned into its underlying fluxes, assimilation (GPP) and respiration (R_{ECO}). To parameterize temperature dependant R_{ECO}, equal to nighttime NEE due to missing assimilation, the Lloyd–Taylor function was applied (Lloyd and Taylor 1994; Falge et al. 2001; Ammann et al. 2007; Reichstein et al. 2005). Light response regression on the basis of the Michaelis–Menten function (Michaelis and Menten 1913) was used to parameterize daytime solar radiation

dependant GPP (Falge et al. 2001; Ruppert et al. 2006). For both, the flux-partitioning model used a time–window scheme instead of the conventional temperature binning approach that was suitable for sites with distinct seasonal variation (Ammann et al. 2007).

^{13}C pulse labeling for determination of relative proportion of C partitioning

Experiment setup

Five stainless steel soil frames (each 1×1 m) with a u-shaped bar at the upper end were inserted up to 10 cm depth, 3 weeks prior to labeling in order to reduce disturbances. For $^{13}\text{CO}_2$ pulse labeling the upper part of the chamber, consisting of aluminum frames (base of the frame 1×1 m, height 0.5 m, cross section 2×2 cm) were placed into the u-shaped bar, which was filled with water (containing a small amount of H_2SO_4) to ensure sealing of upper and lower parts of the chamber. The aluminum frames were covered with transparent LDPE-foil (thickness: 0.2 mm; total light transmission: $\sim 90\%$) shortly before the tracer addition. To minimize the influence of the chamber on the tracer uptake, five cooling aggregates (EZetil Iceakku, 220 g) arranged in parallel were installed in each chamber. A fan positioned behind the aggregates guaranteed turbulent mixing of the chamber air and forced the air to pass the cooling aggregates. High temperatures were thereby avoided and the humidity was reduced by condensation of the water vapor at the cooling aggregates' surfaces. Hence, the condensation at the chamber walls was reduced and better light conditions for the plants were assured. For more detailed information about the chamber construction see Drösler (2005). A flask, containing the ^{13}C tracer as $\text{Na}_2^{13}\text{CO}_3$ (5 g 99% ^{13}C -enriched Na_2CO_3), was placed behind the fan to assure homogenous distribution of the labeled CO_2 . An excess of 5 M H_2SO_4 was added to the tracer solution from outside the chamber through the transparent LDPE-foil with a syringe. The puncture holes in the foil were afterwards sealed with tape. The labeling was done almost simultaneously for all five chambers with only short time shifts of some minutes. Plants were labeled for 3 h to assure complete uptake of the $^{13}\text{CO}_2$. To avoid noon depression of photosynthesis, labeling was conducted from 2:30 to 5:30 pm. In one of the chambers the CO_2 concentration was monitored with an infrared gas analyzer (LI-820, LI-COR Biosciences, Lincoln, Nebraska USA) at the beginning and at the end

of the labeling. The IR-sensor of this device detects only about 30% of the $^{13}\text{CO}_2$ (McDermitt et al. 1993), but the concentration increased at the beginning to a value of about 1400 ppm and a concentration next to zero after the 3 h was measured indicating the complete uptake of the tracer. Shortly before the labeling the CO_2 concentration within the chamber dropped down to zero due to assimilation. It is expected that this very short lack of CO_2 had no noticeable influence on the experiment.

Data acquisition and analysis

Translocation of the assimilated ^{13}C was analyzed during a 21-day period in shoots, roots, soil and soil CO_2 efflux on all 5 plots. Samples were taken immediately (0), 1, 2, 4, 9 and 21 days after the labeling. Shoots were sampled from a circular area of 10 cm diameter. Soil samples were taken in the middle of this area from 0 to 30 cm depth using a soil corer (inner diameter: 4.6 cm). Afterwards, the holes in the soil were plugged with PVC-tubes to avoid changing conditions around the holes.

In addition, samples from unlabeled plots were taken in the same way close to each of the labeled plots to determine the $\delta^{13}\text{C}$ natural abundance for calculations.

All samples were frozen ($-20\text{ }^\circ\text{C}$) until further analysis. Roots were carefully separated from the soil samples with tweezers. All shoot, root and soil samples were dried, weighed and homogenized by ball milling.

Total C and the $\delta^{13}\text{C}$ (‰) signatures of the samples were determined using an element analyzer – isotope ratio mass spectrometer (EA-IRMS, Delta Plus; Thermo Fisher Scientific, Bremen, Germany, interfaced to an elemental analyzer (NC 2500; CE Instruments, Milano, Italy) and calibrated with reference to the international standard VPDB (Vienna Peedee Belemnite).

The total CO_2 efflux from soil was determined on all labeled and on unlabeled (natural abundance) plots with the static alkali (NaOH) absorption method (Lundegardh 1921; Kirita 1971; Singh and Gupta 1977). After cutting the vegetation to avoid any fractionation of the isotopic signal by photosynthesis and shoot respiration, a stainless steel soil collar (i.D. 11 cm; height 10 cm) was placed 5 cm into the soil. It has to be considered that cutting aboveground vegetation may cause decrease in root respiration and increased turnover of dead root biomass. A jar with 1 M NaOH were placed into each collar and the collar was closed with a dark lid.

Soil CO₂ efflux was calculated using the following equation:

$$F_{CO_2,soil} = \frac{x(C)_P}{A \cdot \Delta t} \quad (1)$$

with the total amount of C captured $x(C)_P$ the closed time of the collar Δt and the area enclosed A .

Shortly after the labeling a NaOH trap was placed in each chamber. NaOH was exchanged at each sampling date and additionally on the 12th day after labeling. The amount of NaOH was adjusted to the period by increasing from 40 ml at the beginning up to 80 ml at the end, to be sure that the neutralization did not exceed one-third of the capacity of the NaOH (Gupta and Singh 1977). The amount of collected C was determined by a C/N analyzer (Multi N/C 2100, AnalytikJena, Germany). To obtain $\delta^{13}C$ (‰) values, SrCO₃ was precipitated with SrCl₂, neutralized and dried for the EA-IRMS measurements.

For the calculation of the relative proportion of ¹³C input into various pools (shoots, roots, soil and CO₂ efflux were investigated) after ¹³CO₂ pulse labeling several calculation steps were necessary. The enrichment of ¹³C in a C pool ($x^E(^{13}C)$, atom%) was derived by subtracting the naturally abundant amount of ¹³C ($x(^{13}C)_{std}$, atom%) from the amount of ¹³C in the labeled pool P ($x(^{13}C)_P$, atom%):

$$x^E(^{13}C) = x(^{13}C)_P - x(^{13}C)_{std} \quad (2)$$

where E marks the excess on ¹³C of the atom fraction x (= amount of an isotope of a chemical element, divided by the total amount of atoms of this element; Coplen 2011).

The natural abundance $\delta^{13}C$ value of soil CO₂ efflux, measured beside the labeling plots, was determined by correcting the measured $\delta^{13}C$ values for the admixture of atmospheric CO₂, based on the Miller/Tans model (Miller and Tans 2003; Pausch and Kuzyakov 2012). Therefore, measured $\delta^{13}C$ values multiplied by the respective CO₂ concentrations were plotted against the CO₂ concentrations. The slope of the regression line is equivalent to the $\delta^{13}C$ value of soil CO₂ efflux purified from atmospheric CO₂ (Miller and Tans 2003). The Miller/Tans model was applied in combination with a geometric mean regression (GMR), as suggested for soil CO₂ by Kayler et al. (2010). The standard errors for the slope of the GMR were taken from the respective ordinary least square regression (Sokal and Rohlf 2008).

These standard errors may not completely characterize the uncertainty (Zobitz et al. 2006).

By multiplication with the total C amount ($n(C)_P$, g C m⁻²) of the pool, the ¹³C amount ($n(^{13}C)_P$, g ¹³C m⁻²) of the pool was calculated:

$$n(^{13}C)_P = x^E(^{13}C) \cdot n(C)_P \quad (3)$$

Since all calculations were carried out with area units (m⁻²) it has to be mentioned that in the case of soil and roots all results referred to the sampled soil layer from 0 to 30 cm. To gain a reference value for the recovered amount of ¹³C during the sampling period, the total amounts of ¹³C found immediately after the labeling ($n(^{13}C)_{P_{t_0}}$, g ¹³C m⁻²) were summed up over all investigated pools. Then the ¹³C amounts of every single pool at every point of time ($n(^{13}C)_{P_t}$, g ¹³C m⁻²) could be related to this total value and the recovery (R , %) of the tracer could be calculated using the equation:

$$R(^{13}C)_{P_t} = \frac{n(^{13}C)_{P_t}}{\sum_{i=1}^4 n(^{13}C)_{P_{t_0}(i)}} \quad (4)$$

where t represents any date of sampling and t_0 the point of time immediately after the labeling, when samples were taken for the first time. These calculations were conducted similarly for all pool types i with one exception. In contrast to the other pools, where sampling was destructive and therefore spatially distributed, the ¹³C amount ($n(^{13}C)_P$, g ¹³C m⁻²) within the CO₂ efflux ($F_{CO_2,soil}$) was always sampled at the same position. This was compensated by finally summing all values of the single sampling dates. Hence, the complete amount of ¹³C was considered in that pool as well.

The losses of ¹³C by shoot respiration were not measured, but could be estimated by the following equation:

$$R(^{13}C)_{P_{tShoot}} = 100\% - \sum_{i=1}^4 R(^{13}C)_{P_t(i)} \quad (5)$$

Translocation to deeper soil layers was excluded by taking and analyzing samples between 30 and 50 cm depth. There no noteworthy amount of tracer could be detected. Consequently, it is assumed that shoot respiration is the only relevant missing sink of ¹³C within the considered system, the ¹³C recovered (%) of all four

measured pools i could be summed, and then subtracted from 100 % (Hafner et al. 2012). However, a slight overestimation of the soil respiration might occur due to missing of small amounts of carbon leaching during the rainfall events. To assure that the ^{13}C recovered no longer changed in time, i.e., that the allocation did reach a steady state, the ^{13}C recovery in all pools was checked by applying a repeated measures ANOVA with a *post hoc* Bonferroni test. Means and standard errors of the means (SEM) are presented in the figures and tables.

To finally gain absolute C input into the particular ecosystem pools, labeling and eddy-covariance results were combined, i.e., the relative proportion of the ^{13}C recovered at the end of the C allocation was combined with the total C input into the system

$$n(C)_P = GPP \cdot R(^{13}\text{C})_{P_{\text{end}}} \quad (6)$$

where $n(C)_P$ ($\text{g C m}^{-2} \text{s}^{-1}$) is the absolute C input of the respective pool.

Note that chamber conditions and CO_2 concentrations during labeling may have influenced the photosynthetic rate. Hence, total CO_2 uptake during labeling presumably differed from that measured by EC. However, we assume that the impact of the chamber conditions on relative ^{13}C partitioning within the plant-soil system were negligible because after the short labeling period (3 h) the plants were again exposed to natural conditions.

Results

Absolute atmospheric CO_2 fluxes

Plants started to growth already at the end of February, and the growth period ended in mid-October (Fig. 1). At the beginning, the biomass growth was decelerated by a frost period in March, and during summer the assimilating biomass was harvested by two cutting events, (DOY 188 and 265, marked with ‘c’ in Fig. 1) which became apparent in the GPP and NEE time series.

The isotopic pulse labeling was conducted on June 16th (DOY 167, left edge of grey dashed box in Fig. 1) and the subsequent chase period (CP, grey dashed box in Fig. 1, Table 1), where samples were taken to investigate ^{13}C dynamics and translocation, ended on July 06th (DOY 187) with the last sampling, shortly before the first meadow cutting. The most extreme precipitation

events were measured in August. The fluxes at the labeling day and during the chase period (CP) are shown in Table 1. The mean daily sum of GPP at the labeling day was $-6.0 \text{ g C m}^{-2} \text{ d}^{-1}$ whereas a mean GPP of $-7.1 \pm 0.4 \text{ g C m}^{-2} \text{ d}^{-1}$ was determined for the whole chase period. Figure 1 provides a general view of the intra-annual variability of the ecosystem fluxes, indicating that a number of pulse labeling experiments would be necessary to achieve detailed seasonal partitioning of absolute carbon fluxes. The labeling experiment was conducted within a long time period with a quite uniform assimilation flux that did not end until the first cutting (Fig. 1).

^{13}C dynamics and allocation

The sampling immediately started after the $^{13}\text{CO}_2$ tracer was completely assimilated. The ^{13}C recovery in the shoot biomass strongly decreased from 72.3 % immediately after labeling to 46.6 % 1 day after labeling, mainly due to shoot respiration (Fig. 2). About 14.7 % of ^{13}C was translocated from shoots into roots directly after labeling. The ^{13}C recovery of roots did not change significantly over 21 days. In contrast to the roots, the maximum ^{13}C amount of the soil pool was detected 1 day after the labeling. Thereafter, the ^{13}C recovery in the soil slightly decreased and reached 6.4 % 21 days after labeling (Fig. 2b). Similar to shoot respiration, ^{13}C in soil CO_2 efflux was highest during the first day and then declined over time.

The allocation of ^{13}C tracer was mostly completed after 9 days and the ^{13}C recovery in all pools did not change significantly between the last two samplings. Therefore, the precondition for the partitioning of the absolute C input, the steady state, was fulfilled. Figure 2b illustrates the final percentage at the end of the translocation process. The C flux back into the atmosphere, consisting of shoot respiration and soil CO_2 efflux, dominates the proportion by accounting for almost half (46.7 %) of the assimilated ^{13}C . About one third (34.9 %) remains in the shoots, while roots and soil obtain, with 12 and 6.4 %, respectively, comparatively small proportions of ^{13}C . Overall about 32 % of assimilated ^{13}C were allocated to below-ground pools

Partitioned absolute C allocation

The absolute amount of total assimilated C (GPP) by the ecosystem during the chase period (CP in Table 1) was

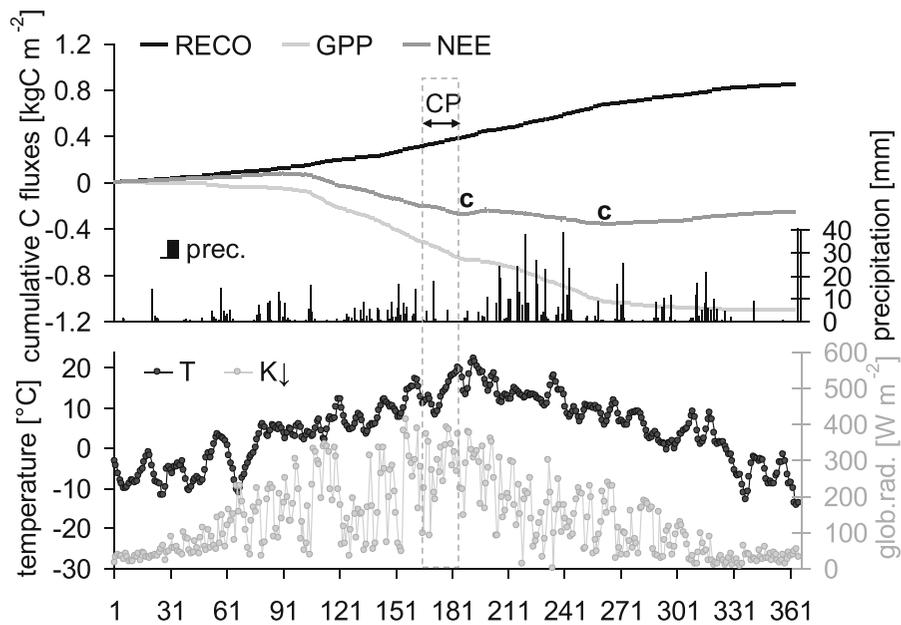


Fig. 1 Cumulative annual fluxes of NEE, GPP and R_{ECO} (flat lines), daily sums of precipitation (black bars), daily means of global radiation (grey filled circles) and daily mean temperatures (black filled circles). The box with dashed outline begins with the

pulse labeling and comprises the chase period (CP), beginning with the pulse labeling and ending shortly before the first mowing event (c). Time on x-axis in day of year (DOY)

partitioned for absolute C allocation into individual pools based on the ^{13}C recovery of the respective pool. The ^{13}C recovery rates could only be applied to the GPP from the chase period (Fig. 1), since the transferability beyond this period was not validated by accounting for, for example plant physiological factors. On average, $2.5 \pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$ were incorporated into the shoot and $0.8 \pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ into the root biomass. $0.5 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ remained in the soil, whereas $2.3 \pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ were released to the atmosphere as shoot respiration and $1.0 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ as soil CO_2 efflux. The sum of the soil CO_2 efflux and shoot respiration ($3.3 \pm 0.4 \text{ g C m}^{-2} \text{ d}^{-1}$) is in accordance with the R_{ECO} of $3.5 \pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$ (Fig. 3). Since R_{ECO} was determined

independently of the labeling by separating the NEE by the FPM, the equal results verify our approach

Discussion

Discussion overview

By combining the results of atmospheric CO_2 flux measurements and $^{13}CO_2$ pulse labeling, a new approach for partitioning ecosystem C fluxes was introduced. In the following, the results will be discussed in detail, i.e., absolute atmospheric CO_2 fluxes will be compared to further flux measurements under similar environmental

Table 1 Annual ($\text{g C m}^{-2} \text{ a}^{-1}$) and daily ($\text{g C m}^{-2} \text{ d}^{-1}$) C fluxes (\pm SEM) for the chase period (CP) and the day of labeling (June 16th) in 2010

	2010 (365 days) annual sum	Chase period (21 days) mean of daily sums	Labeling day daily sum
NEE	-249	-3.5 ± 0.4	-1.8
GPP	-1097	-7.1 ± 0.3	-6.0
R_{ECO}	849	3.5 ± 0.2	4.1
Harvest	158		
Balance	-91		

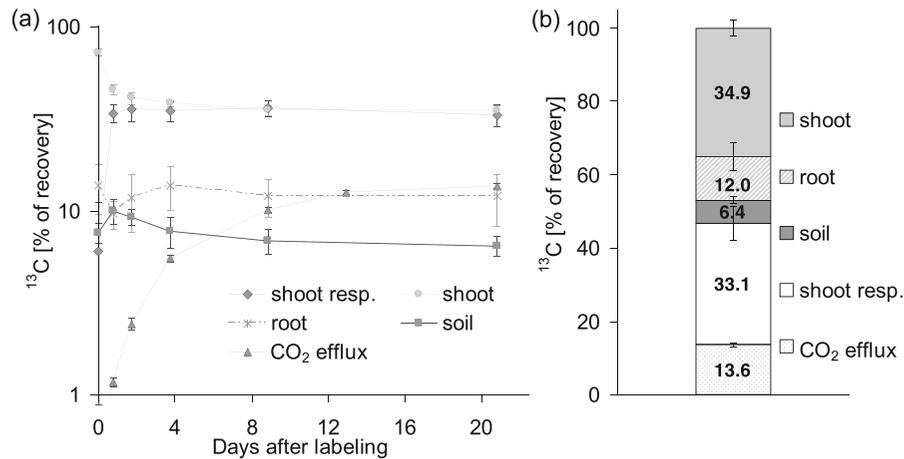


Fig. 2 Cumulative ^{13}C label-incorporation into the various C-pools; **a** ^{13}C dynamics during the chase period; **b** relative proportion of ^{13}C recovered, i.e., final distribution by percentage at the last day of sampling (day 21) in the ecosystem C pools; the

x-axis of (a) intersects at $y=1\%$ for a better illustration. Only one value remains below 1% , the $^{13}\text{CO}_2$ efflux immediately after the labeling accounting for 0.1% . Error bars represent standard errors of the mean ($\pm\text{SEM}$)

conditions, and relative assimilate distribution will be compared to those of other ^{13}C labeling experiments. Since there are no studies referring to comparable efforts in determining partitioned absolute C allocation in the plant–soil–atmosphere system, on-hand results are compared to studies in which these quantities were estimated.

Atmospheric C fluxes

NEE was directly measured by eddy-covariance in 2010 ($-249\text{ g C m}^{-2}\text{ a}^{-1}$). After subtraction of the harvest output ($158\text{ g C m}^{-2}\text{ a}^{-1}$), $-91\text{ g C m}^{-2}\text{ a}^{-1}$ still remained, identifying the site as being a relatively big

carbon sink in relation to other comparable extensively managed grasslands. In Table 2, recent studies dealing with atmospheric CO_2 fluxes on such grasslands at elevations from 375 to 1770 m a.s.l., with mean annual temperatures from 5.5 to $9.5\text{ }^\circ\text{C}$ and annual precipitation sums from 655 to 1816 mm, were reviewed. Although the sites were chosen in a range which was as narrow as possible in terms of these parameters, there are notable differences in the NEE. However, the NEE of the present study lies in the middle of those of the reviewed studies (Table 2). In general, the role of grasslands in the global carbon cycle is still uncertain, as recently described by Gilmanov et al. (2010). There a mean NEE of $70\text{ g C m}^{-2}\text{ a}^{-1}$, but also maximum C sources up to

Fig. 3 Average daily absolute input (GPP), output (R_{ECO}) and partitioned absolute C distribution after assimilation ($\text{g C m}^{-2}\text{ d}^{-1}$, $\pm\text{SEM}$) during the chase period of the labeling experiment. Please note that for illustration all values, even GPP, have a positive sign

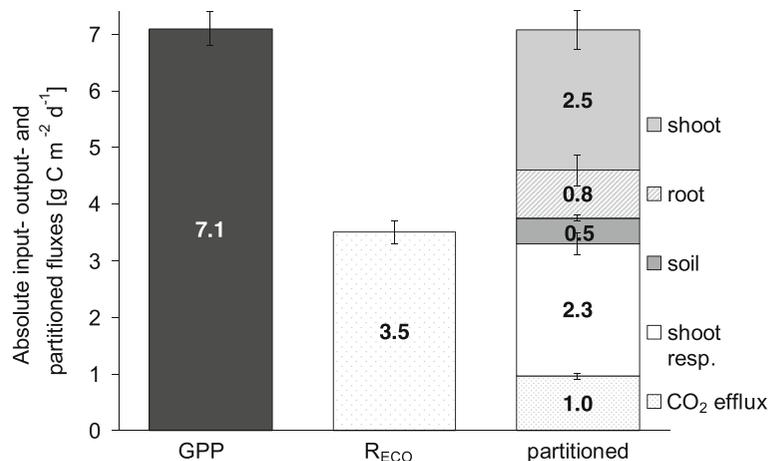


Table 2 Atmospheric C fluxes, determined on European grassland sites with comparable parameters: elevation (m a.s.l.), annual sum of precipitation (RR, mm), sorted by annual mean temperature (T, °C); all sites were managed extensively, some with temporary light grazing instead of cutting. Harvest means harvested C yield from field. All fluxes are presented in $\text{g C m}^{-2} \text{a}^{-1}$

No.	Site	Year	Elevation	T	RR	Management	Harvest	NEE	GPP	R _{ECO}	References
1	Amplero, Italy	2004	900	9.5	1234	extensive, cut / grazed		-214	-1303	1089	(Gilmanov et al. 2007)
2	Oensingen, Switzerland	2002–2004	450	9.0	1109	extensive, 3 cuts	311	-254	-1856	1592	(Ammann et al. 2007)
3	Grillenbug, Germany	2003/2004	375	7.9	655	extensive, 2 and 3 cuts	~147	-278	-1128	851	(Hussain et al. 2011)
4	Laqueille, France	2002–2004	1040	7.0	1200	extensive, grazed		-75	-1514	1440	(Allard et al. 2007)
5	Neustift, Austria	2001–2006	970	6.5	852	extensive, 3 cuts	~317	18	-1568	1586	(Wohlfahrt et al. 2008)
6	Alinyá, Spain	2003/2004	1770	6.1	1064	extensive, grazed		-47	-606	559	(Gilmanov et al. 2007)
7	Voitsumra, Germany	2010	624	5.8	1066	extensive, 2 cuts	158	-249	-1097	849	This study
8	Monte Bondone, Italy	2004	1550	5.5	1189	extensive, 1 cut		-75	-1235	1160	(Gilmanov et al. 2007)
9	Malga Arpaco, Spain	2003	1699	5.5	1816	extensive, grazed		-443	-1083	640	(Gilmanov et al. 2007)

481 $\text{g C m}^{-2} \text{a}^{-1}$ and maximum C sinks up to $-366 \text{ g C m}^{-2} \text{a}^{-1}$ were reviewed for extensively managed grasslands all over the world.

Separating NEE into underlying assimilation (GPP) and respiration (R_{ECO}) fluxes using the short time window scheme was certain to capture the dynamics of this fast changing ecosystem (Ammann et al. 2007; Wohlfahrt et al. 2012), because it sufficiently accounted for seasonal parameter variability (Lasslop et al. 2010). Total annual sums in 2010 (R_{ECO}: 849 and GPP: $-1097 \text{ g C m}^{-2} \text{a}^{-1}$) are within the range of those reviewed in Table 2. It is therefore important to note that the results of this study match best to sites with certain restrictions relating to ecosystem productivity, e.g., low annual temperature means, combined with high elevations (site No. 8 and 9, Table 2). There is also good agreement with another low elevation site (No. 3, Table 2), but in that case GPP is probably limited by a lack of precipitation. With that exception, the grassland in the present study is more comparable to higher elevation sites due to its cold climate. This is also confirmed by R_{ECO}, which is on average smaller than that of the warmer sites with low elevation, but higher than that of high elevation sites. Ammann et al. (2007), who even applied a similar flux partitioning model on an extensively managed grassland in Switzerland, found C fluxes more than one third higher, despite similar elevation and precipitation, but with a 3.2 K higher mean annual temperature.

In a global context, European extensively managed grasslands are outstandingly productive. While Gilmanov et al. (2010) reviewed a worldwide GPP of $-154 \pm 463 \text{ g C m}^{-2} \text{a}^{-1}$, Schulze et al. (2010) found an average GPP for Europe that is almost ten times higher: $-1343 \pm 269 \text{ g C m}^{-2} \text{a}^{-1}$. This in turn is within the range of the GPP of grassland sites reviewed in Table 2, which are obviously representative for European extensively managed grasslands.

Relative ¹³C allocation

Isotopic pulse labeling, the most frequently applied tracer method, was used to quantify the input of ¹³C to diverse ecosystem C pools. At first view, pulse labeling reveals the relative distribution of assimilated C at the moment of labeling and not the distribution of total unlabeled C in different plant parts (Kuzyakov and Domanski 2000). However, by observing ¹³C allocation over a certain period, up to a steady state within the

whole plant-soil-atmosphere system, a representative proportion for total C is finally found (Saggar et al. 1997; Saggar and Hedley 2001; Wu et al. 2010). The end of the chase period was defined as occurring when the amount of ^{13}C recovered in the last two samples of each pool no longer changed significantly (Saggar et al. 1997). That happened after 21 days (cf. Keith et al. 1986; Swinnen et al. 1994). Depending on the pools considered and the sampling frequency, the end of the ^{13}C (^{14}C) allocation period was defined as being between 4 and 28 days (Domanski et al. 2001; Wu et al. 2010; Hafner et al. 2012; Ostle et al. 2000; Saggar et al. 1997). While numerous pulse labeling studies address the back diffusion of tracer to soil pore space occurring during the labeling (Subke et al. 2009; Bahn et al. 2009; Staddon 2003; Leake et al. 2006), dealing with isotopic steady state (after 21 days) allows this difficulty to be disregarded, as it is only relevant for the first 2 days after the labeling (Gamnitzer et al. 2011; Biasi et al. 2012).

In accordance with Wu et al. (2010), the percentage of ^{13}C recovered – rather than the isotope fraction – was used to determine the overall proportion. Calculation of the ^{13}C recovered was achieved by referring to summation of ^{13}C in all measured pools (Kaštovská and Šantrůčková 2007; Hafner et al. 2012) in order to not underestimate the initial fixation by considering only ^{13}C found in shoots directly after labeling. About one third of the C remains in the shoot biomass as reviewed by Kuzyakov and Domanski (2000) for numerous pasture plant studies (Table 3). In contrast, agricultural plants like wheat or maize incorporate a larger proportion (50–60 %) into the shoot (Jones et al. 2009; Table 3). During the chase period the amount of tracer decreased by 48 % within the shoots, which is quite close to the 32–51 % of Johnson et al. (2002) and 55 % of Butler et al. (2004) and Wu et al. (2010). Higher rates are also possible for grasslands, e.g., 77 % (Ostle et al. 2000) and 70 % (Leake et al. 2006), even during the first day after the labeling. In this study the maximum decline also took place between first and second sampling, including the first night after the labeling, caused mainly by night-time shoot respiration and allocation to roots (Butler et al. 2004; Leake et al. 2006). Shoot respiration dynamics agree with this finding, by increasing after the first sampling, which took place in the late afternoon at the labeling day. The much higher percentage of ^{13}C was recovered at the second sampling resulted from the

above-mentioned night-time fluxes. However, shoot respiration dynamics seem feasible and the final proportion of 30 % lies within the range found in the literature (Table 3).

The proportion of below-ground C input (32 %) into roots (12 %), soil (6.4 %) and CO_2 efflux (13.6 %) is also in line with Kuzyakov and Domanski (2000); (Table 3). The relatively low allocation to below-ground pools, especially to the root system, may be explained on the general steadiness of long-established grassland root systems (Saggar et al. 1997). This was confirmed by the biomass data and can be also an explanation for the non-significant changes of the ^{13}C recovery during the chase period (Fig. 2). However, results of other studies are quite heterogeneous, but these found mostly higher amounts (Table 3) and, beyond that, diverse patterns in C allocation to roots. The maximum amount of tracer reached the roots one (Johnson et al. 2002) or 2 days (Ostle et al. 2000; Staddon 2003), or even weeks later, but then mostly without significant differences (Rangel-Castro et al. 2004; Leake et al. 2006; Hafner et al. 2012). A slight peak at the fourth day as in the current study is a realistic result if it is considered that Kuzyakov and Domanski (2000) suggested a period of hours to days after the labeling. CO_2 efflux from soil exhibits the same pattern presented by Staddon (2003) and Hafner et al. (2012): An initial peak, an exponentially decreasing recovery of ^{13}C over time and a decreasing slope in the cumulative $^{13}\text{CO}_2$ efflux (Fig. 2). This pattern of the soil CO_2 efflux indicates fast translocation of recently assimilated C through the system, probably released by root-derived respiration (Kuzyakov et al. 2001). However, CO_2 efflux from soil was determined with the static alkali (NaOH) absorption method. This method is useful but has also disadvantages as e.g., scrubbing CO_2 from the chamber headspace of missing atmospheric turbulence. Although those two are opposite effects, on the whole the flux rate might be overestimated. Compared to the other pools, ^{13}C enrichment of bulk soil after pulse labeling was relatively low. The amount of ^{13}C recovered in the soil (6.4 %) is comparable to other studies, especially those summarized in the reviews (Table 3). A slightly higher amount of ^{13}C was found after 1 day, but just as the weak peaks of Staddon (2003) after 12 and Rangel-Castro et al. (2004) after 7 days, it was not significant (Fig. 2).

Table 3 Comparable partitioning studies related to species, methods and investigated compartments. All values are presented in % of recovery or % of (net) assimilated tracer

No.	Plant / Conditions	Method	Days after labeling	Shoot	Below ground	Root	Soil	R _{ECCO}	Shoot resp.	CO ₂ efflux	References
1	Lolium perenne / controlled cond.	¹⁴ C pulse, % of recovery	7	49.8	40.7	21.8	1.8	16.6	9.5	17.1	(Ratray et al. 1995)
2	Lolium perenne / controlled cond.	¹⁴ C pulse, % of recovery	2	40.0	60.0	14.6	30.0	15.4			(Bazot et al. 2006)
3	Festuca, controlled cond.	¹³ C pulse, % of recovery	2	43.9	54.9	39.7	4.1			11.1	(Allard et al. 2006)
4	White clover / controlled cond.	¹⁴ C pulse, % of recovery	2	56.9	43.0	9.0	7.2			26.8	(Todorovic et al. 1999)
5	Grassland / field conditions	¹³ C pulse, % of recovery	32	28.9	58.7	34.2	7.3	29.6	12.4	17.2	(Wu et al. 2010)
6	Grassland / field conditions	¹³ C pulse, % of recovery	27	38.0	20.0	0.5	10.4	51.0	42.0	9.0	(Hafner et al. 2012)
7	Pasture / field conditions	¹⁴ C pulse, % of recovery	35	26.4		34.7	2.1	36.8			(Saggar et al. 1997)
8	Pasture / field conditions	¹⁴ C pulse, % of recovery	35	31.0		27.0	5.2	37.0			(Saggar and Hedley 2001)
9	Lolium perenne / controlled cond.	¹⁴ C continuous ^a , % of recovery		47.8	52.1	39.7	2.6			9.8	(van Ginkel et al. 1997)
10	Brome grass / controlled cond.	¹⁴ C % rep. pulse ^b , % of assimilated ^c		27.0		5.0	14.0	54.0			(Davenport and Thomas 1988)
11	21 agric.plants Review	% of net assimilated ^d		60	36	19	5			12	(Jones et al. 2009)
12	Pasture plants, Review	% of assimilated ^c		30	40	20	5	45	30	15	(Kuzakov and Domanski 2000)
13	Grassland / field conditions	¹³ C pulse, % of recovery	21	34.9	32.0	12.0	6.4	46.7	33.1	13.6	this study

^a Continuously labeled with ¹⁴C during the whole time of growing

^b Weekly repeated pulse labeling, as suggested to cover whole growth period

^c Recovery related to assimilated amount of tracer (shoot respiration is not considered, may underestimate final result by up to 30 %)

^d Recovery related to net assimilated amount of tracer (shoot respiration is considered)

Partitioned absolute C fluxes

Up to now, partitioned absolute amounts of allocated C were only roughly estimated, although in most studies addressing to C balance and turnover, total masses are important. Kuzyakov and Domanski (2000) calculated mean absolute values for below-ground translocated C by grasses and cereals from the literature: 179 g C m^{-2} for all studies and 220 g C m^{-2} for studies longer than 100 days (i.e., 2.2 g C m^{-2} on average). Absolute C inputs found for an alpine *Kobresia humilis* pasture (Wu et al. 2010) were about one third smaller than in the present study in all compartments except the roots, when taking the length of the growth period into account. This results from the generally lower turnover rates in high altitude grasslands (Budge et al. 2011). In addition to that, the percentage of root biomass is considerably higher in these regions (Ammann et al. 2009; Leifeld et al. 2009; Unteregelsbacher et al. 2011). One further comparison allows the rough estimation of total C in- and outputs for pasture plants. Kuzyakov and Domanski (2000) measured fluxes that are on average 1.5 times lower than that of this study, but the input into the root system matches very well.

Obviously, there is a lack of studies presenting absolute values of C input to distinct ecosystem compartments. Coupling of atmospheric C flux measurements with ^{13}C pulse labeling provides partitioning of absolute C fluxes. In general, the combination of methods works well and allows a more detailed insight into the C cycle of grasslands. One limitation is that the expansion beyond the chase period has to be checked independently using other methods. Whereas the atmospheric fluxes are mostly representative, at least as long as weather conditions and management activities are within a certain range, plant physiological parameters – and thereby partitioning patterns – vary too much to allow transfer of the result of a single pulse labeling to the whole growth period (Gregory and Atwell 1991; Kuzyakov and Domanski 2000). In contrast, a series of labeling pulses at regular intervals (Keith et al. 1986; Swinnen et al. 1994; Kuzyakov et al. 1999, 2001; Kuzyakov and Schneckenberger 2004; Davenport and Thomas 1988) could provide reasonable estimates of the relative partitioning for the whole growth period, to be applied to the more easily available time series of C input, obtained by micrometeorological flux measurements. This way, mowing events or grazing could also be considered.

Concluding remarks

Application of EC showed that the extensively managed grassland was a significant net carbon sink of $-91 \text{ g C m}^{-2} \text{ a}^{-1}$ in 2010. The NEE flux-partitioning model revealed a mean underlying assimilated amount of carbon of $-7.1 \pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ during the 21 days of the ^{13}C pulse labeling experiment. Pulse labeling and tracing provided relative partitioning of ^{13}C input into distinct ecosystem C pools. First-time combining the results of these methods to an integrative approach allowed partitioning of absolute C input by assimilation into absolute C fluxes into shoots, roots and soil and the contributions to the respiration fluxes CO_2 -efflux and shoot respiration. Two different areas benefit from this innovation: labeling approaches are upgraded by finally dealing with absolute instead of relative C allocation and further separation of the NEE beyond assimilation and respiration fluxes is provided. Moreover, individual reactions of sensitive subsidiary ecosystem pools and processes can be detected and evaluated on the basis of mass units.

However, under the currently changing environmental conditions, both approaches benefit from the reduction of uncertainties by the detection and evaluation of individual reactions of sensitive subsidiary ecosystem pools and processes on the basis of mass units. The results of this study are in line with the available literature and should encourage combining methods of atmosphere, plant and soil science also in future studies. The suggested method can be also applied to C pools such as microbial biomass and dissolved organic carbon. Also for ecosystem modelers dealing with C pools and fluxes, it provides data on C incorporation in pools in absolute units.

Acknowledgments The project “Investigation of carbon turnover of grasslands in a northern Bavarian low mountain range under extreme climate conditions” was funded within the joint research project “FORCAST” by the Bavarian State Ministry of Sciences, Research and Arts. The authors wish to acknowledge the support of the participants of the FORCAST project, especially Prof. Dr. Anke Jentsch and Alexander Ulmer for evaluation of biodiversity and species determination on our site. Finally, the authors want to thank Prof. Dr. Gerhard Gebauer and his team of the Laboratory of Isotope Biogeochemistry for the abundance analysis of the carbon isotopes, all colleagues, technicians and research assistants who took part in the field and laboratory work, especially Martin Rimpler, Martin Pannek, Ilse Thaufelder, Johannes Olesch and last but not least Peng Zhao for his support with the flux partitioning model.

References

- Allard V, Robin C, Newton P, Lieffering M, Soussana J (2006) Short and long-term effects of elevated CO₂ on *Lolium perenne* rhizodeposition and its consequences on soil organic matter turnover and plant N yield. *Soil Biol Biochem* 38: 1178–1187
- Allard V, Soussana J, Falcimagne R, Berbigier P, Bonnefond J, Ceschia E, D'hour P, Hénault C, Laville P, Martin C, Pinarès-Patino C (2007) The role of grazing management for the net biome productivity and greenhouse gas budget (CO₂, N₂O and CH₄) of semi-natural grassland. *Agric Ecosyst Environ* 121:47–58
- Ammann C, Flechard CR, Leifeld J, Neftel A, Fuhrer J (2007) The carbon budget of newly established temperate grassland depends on management intensity. The greenhouse gas balance of grasslands in Europe. *Agric Ecosyst Environ* 121:5–20
- Ammann C, Spirig C, Leifeld J, Neftel A (2009) Assessment of the nitrogen and carbon budget of two managed temperate grassland fields. *Agric Ecosyst Environ* 133:150–162
- Aubinet M, Grelle A, Ibrom A, Rannik Ü, Moncrieff J, Foken T, Kowalski AS, Martin PH, Berbigier P, Bernhofer C, Clement R, Elbers JA, Granier A, Grünwald T, Morgenstern K, Pilegaard K, Rebmann C, Snijders W, Valentini R, Vesala T (2000) Estimates of the Annual Net Carbon and Water Exchange of Forests: The EUROFLUX Methodology. *Adv Ecol Res*: 113–175
- Aubinet M, Vesala T, Papale D (2012) Eddy covariance. Springer, Dordrecht
- Bahn M, Schmitt M, Siegwolf R, Richter A, Brüggemann N (2009) Does photosynthesis affect grassland soil-respired CO₂ and its carbon isotope composition on a diurnal time-scale? *New Phytol* 182:451–460
- Baldocchi DD (2003) Assessing the eddy covariance technique for evaluating carbon dioxide exchange rates of ecosystems: past, present and future. *Glob Chang Biol* 9:479–492
- Baldocchi DD, Falge E, Gu LH, Olson R, Hollinger DY, Running S, Anthoni P, Bernhofer C, Davis K, Evans R, Fuentes J, Goldstein AH, Katul GG, Law BE, Lee XH, Malhi Y, Meyers TP, Munger JW, Oechel W, Paw UKT, Pilegaard K, Schmid HP, Valentini R, Verma S, Vesala T, Wilson K, Wofsy S (2001) FLUXNET: a new tool to study the temporal and spatial variability of ecosystem-scale carbon dioxide, water vapor, and energy flux densities. *Bull Am Meteorol Soc* 82: 2415–2434
- Bazot S, Ulf L, Blum H, Nguyen C, Robin C (2006) Effects of elevated CO₂ concentration on rhizodeposition from *Lolium perenne* grown on soil exposed to 9 years of CO₂ enrichment. *Soil Biol Biochem* 38:729–736
- Biasi C, Pitkämäki AS, Tavi NM, Koponen HT, Martikainen PJ (2012) An isotope approach based on ¹³C pulse-chase labelling vs. the root trenching method to separate heterotrophic and autotrophic respiration in cultivated peat lands. *Boreal Environ Res* 17(3/4):184–192
- Bowling DR, Tans PP, Monson RK (2001) Partitioning net ecosystem carbon exchange with isotopic fluxes of CO₂. *Glob Chang Biol* 7:127–145
- Buchmann N (2000) Biotic and abiotic factors controlling soil respiration rates in *Picea abies* stands. *Soil Biol Biochem* 32:1625–1635
- Buchmann N (2002) Plant ecophysiology and forest response to global change. *Tree Physiol*: 1177–1184
- Budge K, Leifeld J, Hiltbrunner E, Fuhrer J (2011) Alpine grassland soils contain large proportion of labile carbon but indicate long turnover times. *Biogeosciences* 8:1911–1923
- Butler JL, Bottomley PJ, Griffith SM, Myrold DD (2004) Distribution and turnover of recently fixed photosynthate in ryegrass rhizospheres. *Soil Biol Biochem* 36:371–382
- Chou WW, Silver WL, Jackson RD, Thompson AW, Allen-Diaz B (2008) The sensitivity of annual grassland carbon cycling to the quantity and timing of rainfall. *Glob Chang Biol* 14: 1382–1394
- Ciais P, Reichstein M, Viovy N, Granier A, Ogee J, Allard V, Aubinet M, Buchmann N, Bernhofer C, Carrara A, Chevallier F, de Noblet N, Friend AD, Friedlingstein P, Grünwald T, Heinesch B, Keronen P, Knohl A, Krinner G, Loustau D, Manca G, Matteucci G, Miglietta F, Ourcival JM, Papale D, Pilegaard K, Rambal S, Seufert G, Soussana JF, Sanz MJ, Schulze ED, Vesala T, Valentini R (2005) Europe-wide reduction in primary productivity caused by the heat and drought in 2003. *Nature* 437:529–533
- Ciais P, Soussana JF, Vuichard N, Luysaert S, Don A, Janssens IA, Piao SL, Dechow R, Lathière J, Maignan F, Wattenbach M, Smith P, Ammann C, Freibauer A, Schulze ED (2010) The greenhouse gas balance of European grasslands. *Biogeosci Discuss* 7:5997–6050
- Coplen TB (2011) Guidelines and recommended terms for expression of stable-isotope-ratio and gas-ratio measurement results. *Rapid Commun Mass Spectrom* 25:2538–2560
- Davenport JR, Thomas RL (1988) Carbon partitioning and rhizodeposition in corn and bromegrass. *Can J soil Sci*: 693–701
- Davidson EA, Savage K, Verchot LV, Navarro R (2002) Minimizing artefacts and biases in chamber-based measurements of soil respiration. *FLUXNET 2000 Synthesis. Agric For Meteorol* 113:21–37
- Desai AR, Richardson AD, Moffat AM, Kattge J, Hollinger DY, Barr A, Falge E, Noormets A, Papale D, Reichstein M, Stauch VJ (2008) Cross-site evaluation of eddy covariance GPP and RE decomposition techniques. *Agric For Meteorol* 148:821–838
- Domanski G, Kuzyakov Y, Siniakina SV, Stahr K (2001) Carbon flows in the rhizosphere of ryegrass (*Lolium perenne*). *J Plant Nutr Soil Sci* 164:381–387
- Dore S, Hymus GJ, Johnson DP, Hinkle CR, Valentini R, Drake BG (2003) Cross validation of open-top chamber and eddy covariance measurements of ecosystem CO₂ exchange in a Florida scrub-oak ecosystem. *Glob Chang Biol* 9:84–95
- Drösler M (2005) Trace gas exchange and climatic relevance of bog ecosystems, Southern Germany. PhD-thesis, Technische Universität München, 179 pp. Online publication: urn:nbn:de:bvb:91-diss20050901-1249431017
- Eigenmann R, Metzger S, Foken T (2009) Generation of free convection due to changes of the local circulation system. *Atmos Chem Phys* 9:8587–8600
- Falge E, Baldocchi DD, Olson R, Anthoni P, Aubinet M, Bernhofer C, Burba G, Ceulemans R, Clement R, Dolman H, Granier A, Gross P, Grünwald T, Hollinger DY, Jensen N, Katul GG, Keronen P, Kowalski AS, Lai CT, Law BE, Meyers TP, Moncrieff J, Moors E, Munger JW, Pilegaard K, Rannik Ü, Rebmann C, Suyker A, Tenhunen JD, Tu KP,

- Verma S, Vesala T, Wilson K, Wofsy S (2001) Gap filling strategies for defensible annual sums of net ecosystem exchange. *Agric For Meteorol* 107:43–69
- Falge E, Baldocchi D, Tenhunen J, Aubinet M, Bakwin P, Berbigier P, Bernhofer C, Burba G, Clement R, Davis KJ, Elbers JA, Goldstein AH, Grelle A, Granier A, Guðmundsson J, Hollinger D, Kowalski AS, Katul G, Law BE, Malhi Y, Meyers T, Monson RK, Munger J, Oechel W, Paw UKT, Pilegaard K, Rannik Ü, Rebmann C, Suyker A, Valentini R, Wilson K, Wofsy S (2002) Seasonality of ecosystem respiration and gross primary production as derived from FLUXNET measurements. *Agric For Meteorol* 113:53–74
- Foken T (2003) Lufthygienisch–bioklimatische Kennzeichnung des oberen Egertales (Fichtelgebirge bis Karlovy Vary). *Bayreuther Forum Ökologie*: 70 S
- Foken T, Wichura B (1996) Tools for quality assessment of surface based flux measurements. *Agric For Meteorol* 78:83–105
- Foken T, Göckede M, Mauder M, Mahrt L, Amiro BD, Munger JW (2004) Post–field data quality control. In: Lee X, Massman W, Law B (eds) *Handbook of micrometeorology: a guide for surface flux measurement and analysis*. Kluwer, Dordrecht, pp 181–208
- Foken T, Leuning R, Oncley SP, Mauder M, Aubinet M (2012) Corrections and data quality. In: Aubinet M, Vesala T, Papale D (eds) *Eddy covariance: a practical guide to measurement and data analysis*. Springer, Dordrecht, pp 85–131
- Gammitzer U, Moyes AB, Bowling DR, Schnyder H (2011) Measuring and modelling the isotopic composition of soil respiration: insights from a grassland tracer experiment. *Biogeosciences* 8:1333–1350
- Gavrichkova O (2009) Drivers of soil respiration of root and microbial origin in grasslands. PhD–Thesis, Università degli Studi della Tuscia. http://dspace.unitus.it/bitstream/2067/1104/1/ogavrichkova_tesid.pdf
- Gilmanov TG, Soussana JF, Aires L, Allard V, Ammann C, Balzarolo M, Barcza Z, Bernhofer C, Campbell CL, Cernusca A, Cescatti A, Clifton–Brown J, Dirks BOM, Dore S, Eugster W, Fuhrer J, Gimeno C, Grünwald T, Haszpra L, Hensen A, Ibrom A, Jacobs AFG, Jones MB, Lanigan G, Laurila T, Lohila A, Manca G, Marcolla B, Nagy Z, Pilegaard K, Pinter K, Pio C, Raschi A, Rogiers N, Sanz MJ, Stefani P, Sutton MA, Tuba Z, Valentini R, Williams ML, Wohlfahrt G (2007) Partitioning European grassland net ecosystem CO₂ exchange into gross primary productivity and ecosystem respiration using light response function analysis. *Agric Ecosyst Environ* 121:93–120
- Gilmanov TG, Aires L, Barcza Z, Baron VS, Beletti L, Beringer J, Billesbach D, Bonal D, Bradford J, Ceschia E, Cook D, Corradi C, Frank A, Gianelle D, Gimeno C, Grünwald T, Guo H, Hanan N, Haszpra L, Heilman J, Jacobs AFG, Jones MB, Johnson DA, Kiely G, Li S, Magliulo V, Moors E, Nagy Z, Nasyrov M, Owensby C, Pinter K, Pio C, Reichstein M, Sanz MJ, Scott R, Soussana JF, Stoy PC, Svejcar T, Tuba Z, Zhou G (2010) Productivity, respiration, and light–response parameters of world grassland and agroecosystems derived from flux–tower measurements. *Rangel Ecol Manag* 63:16–39
- Göckede M, Rebmann C, Foken T (2004) A combination of quality assessment tools for eddy covariance measurements with footprint modelling for the characterisation of complex sites. Flux and concentration footprints. *Agric For Meteorol* 127:175–188
- Göckede M, Markkanen T, Hasager CB, Foken T (2006) Update of a footprint–based approach for the characterization of complex measurement sites. *Bound–Layer Meteorol* 118: 635–655
- Goulden ML, Munger JW, Fan S, Daube BC, Wofsy S (1996) Measurements of carbon sequestration by long–term eddy covariance: methods and a critical evaluation of accuracy. *Glob Chang Biol* 2:169–182
- Gregory PJ, Atwell BJ (1991) The fate of carbon in pulse–labelled crops of barley and wheat. *Plant Soil* 136:205–213
- Gupta SR, Singh JS (1977) Effect of alkali concentration, volume and absorption area on the measurement of soil respiration in a tropical sward. *Pedobiologia*: 233–239
- Hafner S, Unteregelsbacher S, Seeber E, Lena B, Xu X, Li X, Guggenberger G, Miehe G, Kuzyakov Y (2012) Effect of grazing on carbon stocks and assimilate partitioning in a Tibetan montane pasture revealed by ¹³C₂ pulse labeling. *Glob Chang Biol* 18:528–538
- Hussain M, Grünwald T, Tenhunen J, Li Y, Mirzae H, Bernhofer C, Otieno D, Dinh N, Schmidt M, Waringer M, Owen K (2011) Summer drought influence on CO₂ and water fluxes of extensively managed grassland in Germany. *Agric Ecosyst Environ* 141:67–76
- IPCC (2007) *Climate change 2007 – the physical science basis. Contribution of working group I to the fourth assessment report of the IPCC*. Cambridge University Press, Cambridge, 996 S
- IUSS Working Group WRB (2007) *World reference base for soil resources 2006 first update 2007*. World soil resources reports no. 103. FAO, Rome
- Janssens IA (2003) Europe’s terrestrial biosphere absorbs 7 to 12 % of European anthropogenic CO₂ emissions. *Science* 300:1538–1542
- Johnson D, Leake JR, Ostle NJ, Ineson P, Read DJ (2002) In situ ¹³C₂ pulse–labelling of upland grassland demonstrates a rapid pathway of carbon flux from arbuscular mycorrhizal mycelia to the soil. *New Phytol* 153:327–334
- Jones DL, Nguyen C, Finlay RD (2009) Carbon flow in the rhizosphere: carbon trading at the soil–root interface. *Plant Soil* 321:5–33
- Joos O, Hagedorn F, Heim A, Gilgen AK, Schmidt MWI, Siegwolf RTW, Buchmann N (2010) Summer drought reduces total and litter–derived soil CO₂ effluxes in temperate grassland – clues from a ¹³C litter addition experiment. *Biogeosciences* 7:1031–1041
- Kaštovská E, Šantrůčková H (2007) Fate and dynamics of recently fixed C in pasture plant–soil system under field conditions. *Plant Soil* 300:61–69
- Kayler Z, Ganio L, Hauck M, Pypker TG, Sulzman EW, Mix AC, Bond B.J (2010) Bias and uncertainty of δ¹³C₂ isotopic mixing models. *Oecologia*: 227–234
- Keith H, Oades JM, Martin JK (1986) Input of carbon to soil from wheat plants. *Soil Biol Biochem* 18:445–449
- Kirita H (1971) Re–examination of the absorption method of measuring soil respiration under field conditions III. Combined effect of the covered ground area and the surface area of KOH solution on CO₂–absorption rates. *J J Ecol*: 37–42
- Knapp AK (2002) Rainfall variability, carbon cycling, and plant species diversity in a mesic grassland. *Science* 298:2202–2205

- Kuzyakov Y (2006) Sources of CO₂ efflux from soil and review of partitioning methods. *Soil Biol Biochem* 38:425–448
- Kuzyakov Y, Domanski G (2000) Carbon input by plants into the soil. *Review. J Plant Nutr Soil Sci*: 421–431
- Kuzyakov Y, Schneckenberger K (2004) Review of estimation of plant rhizodeposition and their contribution to soil organic matter formation. *Arch Agron Soil Sci* 50:115–132
- Kuzyakov Y, Kretschmar A, Stahr K (1999) Contribution of *Lolium perenne* rhizodeposition to carbon turnover of pasture soil. *Plant Soil* 213:127–136
- Kuzyakov Y, Ehrensberger H, Stahr K (2001) Carbon partitioning and below-ground translocation by *Lolium perenne*. *Soil Biol Biochem* 33:61–74
- Lasslop G, Reichstein M, Papale D, Richardson AD, Ameth A, Barr A, Stoy PC, Wohlfahrt G (2010) Separation of net ecosystem exchange into assimilation and respiration using a light response curve approach: critical issues and global evaluation. *Glob Chang Biol* 16:187–208
- Leake JR, Ostle NJ, Rangel-Castro I, Johnson D (2006) Carbon fluxes from plants through soil organisms determined by field ¹³CO₂ pulse-labelling in an upland grassland. *Appl Soil Ecol* 33:152–175
- Leifeld J, Zimmermann M, Fuhrer J, Conen F (2009) Storage and turnover of carbon in grassland soils along an elevation gradient in the Swiss Alps. *Glob Chang Biol* 15:668–679
- Li M, Babel W, Tanaka K, Foken T (2013) Note on the application of planar-fit rotation for non-omnidirectional sonic anemometers. *Atmos Meas Techn*: 221–229
- Lloyd J, Taylor J (1994) On the temperature dependence of soil respiration. *Funct. Ecol.*: 315–323
- Lundegårdh H (1921) Ecological studies in the assimilation of certain forest plants and shore plants. *Svensk Botaniska Tidskrift*: 46–94
- Luo Y (2007) Terrestrial carbon-cycle feedback to climate warming. *Annu Rev Ecol Evol Syst* 38:683–712
- Luo Y, Hui D, Zhang D (2006) Elevated CO₂ stimulates net accumulations of carbon and nitrogen in land ecosystems: a meta-analysis. *Ecology* 87:53–63
- Mauder M, Foken T (2004) Documentation and instruction manual of the eddy covariance software package TK2. Univ. Bayreuth, Abt. Mikrometeorol., ISSN: 1614–89166, 26, 42 pp
- Mauder M, Foken T (2011) Documentation and instruction manual of the eddy covariance software package TK3. Universität Bayreuth, Abt. Mikrometeorologie, Print, ISSN 1614–8916, Univ. Bayreuth, Abt. Mikrometeorol., ISSN: 1614–89166, 46, 58 pp
- Mauder M, Liebenthal C, Göckede M, Leps J, Beyrich F, Foken T (2006) Processing and quality control of flux data during LITFASS–2003. *Bound-Layer Meteorol* 121:67–88
- Mauder M, Oncley SP, Vogt R, Weidinger T, Ribeiro L, Bernhofer C, Foken T, Kohsiek W, Bruin HAR, Liu H (2007) The energy balance experiment EBEX–2000. Part II: intercomparison of eddy-covariance sensors and post-field data processing methods. *Bound-Layer Meteorol* 123:29–54
- Mauder M, Foken T, Clement R, Elbers JA, Eugster W, Grünwald T, Heusinkveld B, Kolle O (2008) Quality control of CarboEurope flux data – part 2: inter-comparison of eddy-covariance software. *Biogeosciences* 5:451–462
- McDermitt DK, Welles JM, Eckles RD (1993) Effects of temperature, pressure and water vapor on gas phase infrared absorption by CO₂. application note # 116. Li–COR, Lincoln
- Michaelis L, Menten ML (1913) Die Kinetik der Invertinwirkung. *Kinetics of the invertin reaction. Biochemische Zeitschrift*: 333–369
- Miller J, Tans PP (2003) Calculating isotopic fractionation from atmospheric measurements at various scales. *Tellus B*: 207–214
- Moffat AM, Papale D, Reichstein M, Hollinger DY, Richardson AD, Barr AG, Beckstein C, Braswell BH, Churkina G, Desai AR, Falge E, Gove JH, Heimann M, Hui D, Jarvis AJ, Kattge J, Noormets A, Stauch VJ (2007) Comprehensive comparison of gap-filling techniques for eddy covariance net carbon fluxes. *Agric For Meteorol* 147:209–232
- Moncrieff J, Valentini R, Greco S, Seufert G, Ciccioli P (1997) Trace gas exchange over terrestrial ecosystems: methods and perspectives in micrometeorology. *J Exp Bot* 48:1133–1142
- Nguyen C (2009) Rhizodeposition of organic C by plant: mechanisms and controls. *J Sustain Agric*: 97–123
- Ostle NJ, Ineson P, Benham D, Sleep D (2000) Carbon assimilation and turnover in grassland vegetation using an in situ ¹³CO₂ pulse labelling system. *Rapid Commun Mass Spectrom* 14:1345–1350
- Papale D (2012) Data gap filling. In: Aubinet M, Vesala T, Papale D (eds) *Eddy covariance: a practical guide to measurement and data analysis*. Springer, Dordrecht, pp 159–172
- Pausch J, Kuzyakov Y (2012) Soil organic carbon decomposition from recently added and older sources estimated by $\delta^{13}\text{C}$ values of CO₂ and organic matter. *Soil Biol Biochem*: 40–47
- Rangel-Castro I, Prosser JI, Scrimgeour CM, Smith P, Ostle NJ, Ineson P, Meharg A, Killham K (2004) Carbon flow in an upland grassland: effect of liming on the flux of recently photosynthesized carbon to rhizosphere soil. *Glob Chang Biol* 10:2100–2108
- Rannik Ü, Sogachev A, Foken T, Göckede M, Kljun N, Leclerc MY, Vesala T (2012) Footprint analysis. In: Aubinet M, Vesala T, Papale D (eds) *Covariance: a practical guide to measurement and data analysis*. Springer, Netherlands, pp 211–261
- Ratray EAS, Paterson E, Killham K (1995) Characterisation of the dynamics of C-partitioning within *Lolium perenne* and to the rhizosphere microbial biomass using ¹⁴C pulse chase. *Biol Fertil Soils* 19:280–286
- Reichstein M, Falge E, Baldocchi D, Papale D, Aubinet M, Berbigier P, Bernhofer C, Buchmann N, Gilmanov T, Granier A, Grünwald T, Havrankova K, Ilvesniemi H, Janous D, Knohl A, Laurila T, Lohila A, Loustau D, Matteucci G, Meyers T, Miglietta F, Ourcival J, Pumpanen J, Rambal S, Rotenberg E, Sanz M, Tenhunen J, Seufert G, Vaccari F, Vesala T, Yakir D, Valentini R (2005) On the separation of net ecosystem exchange into assimilation and ecosystem respiration: review and improved algorithm. *Glob Chang Biol* 11:1424–1439
- Reichstein M, Stoy PC, Desai AR, Lasslop G, Richardson AD (2012) Partitioning of net fluxes. In: Aubinet M, Vesala T, Papale D (eds) *Eddy covariance: a practical guide to measurement and data analysis*. Partitioning of net fluxes. Springer, Dordrecht, pp 263–289
- Rochette P, Hutchinson GL (2005) Measurement of soil respiration in situ: chamber techniques. In: Hatfield JL, Baker JM

- (eds) *Micrometeorology in agricultural systems*. American Society of Agronomy; Crop Science Society of America; Soil Science Society of America, Madison
- Ruppert J, Mauder M, Thomas C, Lüers J (2006) Innovative gap-filling strategy for annual sums of CO₂ net ecosystem exchange. *Agric For Meteorol* 138:5–18
- Saggar S, Hedley C (2001) Estimating seasonal and annual carbon inputs, and root decomposition rates in a temperate pasture following field ¹⁴C pulse-labelling. *Plant Soil* 236:91–103
- Saggar S, Hedley C, Mackay AD (1997) Partitioning and translocation of photosynthetically fixed ¹⁴C in grazed hill pastures. *Biol Fertil Soils* 25:152–158
- Schulze ED, Ciais P, Luysaert S, Schrupf M, Janssens IA, Thiruchittampalam B, Theloke J, Saurat M, Bringer S, Lelieveld J, Lohila A, Rebmann C, Jung M, Bastviken D, Abril G, Grassi G, Leip A, Freibauer A, Kutsch W, Don A, Nieschulze J, Börner A, Gash JH, Dolman AJ (2010) The European carbon balance. Part 4: integration of carbon and other trace-gas fluxes. *Glob Chang Biol* 16:1451–1469
- Singh J, Gupta SR (1977) Plant decomposition and soil respiration in terrestrial eco-systems. *Bot Rev*: 450–511
- Sokal RR, Rohlf FJ (2008) *Biometry*, 4th edn. W.H. Freeman, New York
- Staddon PL (2003) The speed of soil carbon throughput in an upland grassland is increased by liming. *J Exp Bot* 54:1461–1469
- Stoy PC, Katul GG, Siqueira MB, Juang J, Novick KA, Uebelherr JM, Oren R (2006) An evaluation of models for partitioning eddy covariance-measured net ecosystem exchange into photosynthesis and respiration. *Agric For Meteorol* 141:2–18
- Subke J, Tenhunen JD (2004) Direct measurements of CO₂ flux below a spruce forest canopy. *Agric For Meteorol* 126:157–168
- Subke J, Vallack HW, Magnusson T, Keel SG, Metcalfe DB, Högberg P, Ineson P (2009) Short-term dynamics of abiotic and biotic soil ¹³CO₂ effluxes after in situ ¹³CO₂ pulse labelling of a boreal pine forest. *New Phytol* 183:349–357
- Swinnen J, van Veen JA, Merckx R (1994) ¹⁴C pulse-labelling of field-grown spring wheat: an evaluation of its use in rhizosphere carbon budget estimations. *Soil Biol Biochem* 26:161–170
- Todorovic C, Nguyen C, Robin C, Guckert A (1999) ¹⁴C-assimilate partitioning within white clover plant-soil system: effects of photoperiod/temperature treatments and defoliation. *Eur J Agron* 11:13–21
- Unteregelsbacher S, Hafner S, Guggenberger G, Miede G, Xu X, Liu J, Kuzyakov Y (2011) Response of long-, medium- and short-term processes of the carbon budget to overgrazing-induced crusts in the Tibetan Plateau. *Biogeochemistry*
- van Ginkel J, Gorissen A, van Veen JA (1997) Carbon and nitrogen allocation in *Lolium perenne* in response to elevated atmospheric CO₂ with emphasis on soil carbon dynamics. *Plant Soil* 188:299–308
- Vesala T, Kljun N, Rannik Ü, Rinne J, Sogachev A, Markkanen T, Sabelfeld K, Foken T, Leclerc M (2008) Flux and concentration footprint modelling: state of the art. *Environ Pollut* 152: 653–666
- Werth M, Kuzyakov Y (2008) Root-derived carbon in soil respiration and microbial biomass determined by ¹⁴C and ¹³C. *Soil Biol Biochem* 40(3):625–637. doi:10.1016/j.soilbio.2007.09.022
- Whipps JM (1990) Carbon economy. In: Lynch JM (ed) *The rhizosphere*. John Wiley, Chichester, pp 59–97
- Wichura B (2009) Untersuchungen zum Kohlendioxid-Austausch über einem Fichtenwaldbestand auf der Grundlage von Hyperbolic-Relaxed-Eddy-Accumulation Messungen für das stabile Kohlenstoffisotop ¹³C und von Wavelletanalysen. Dissertation. Bayreuther Forum Ökologie, Band 114, 324 S
- Wohlfahrt G, Hammerle A, Haslwanter A, Bahn M, Tappeiner U, Cernusca A (2008) Seasonal and inter-annual variability of the net ecosystem CO₂ exchange of a temperate mountain grassland: effects of weather and management. *J Geophys Res* 113:1–14
- Wohlfahrt G, Klumpp K, Soussana JF (2012) Eddy covariance measurements over grasslands. In: Aubinet M, Vesala T, Papale D (eds) *Eddy covariance: a practical guide to measurement and data analysis*. Springer, Dordrecht, pp 333–344
- Wu Y, Tan H, Deng Y, Wu J, Xu X, Wang Y, Tang Y, Higashi T, Cui X (2010) Partitioning pattern of carbon flux in a Kobresia grassland on the Qinghai-Tibetan Plateau revealed by field ¹³C pulse-labeling. *Glob Chang Biol* 16:2322–2333
- Yakir D, Sternberg LSL (2000) The use of stable isotopes to study ecosystem gas exchange. *Oecologia* 123:297–311
- Zobitz J, Keener JP, Schnyder H, Bowling DR (2006) Sensitivity analysis and quantification of uncertainty for isotopic mixing relationships in carbon cycle research. *Agric For Meteorol*: 56–75