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Carbon fluxes of an extensive meadow
and attempts for flux partitioning

MICHAEL RIEDERER

Diplom Geoökologe

born 5 October, 1981

in Tirschenreuth, Germany

supervised by

Prof. Dr. Thomas Foken

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Was vor uns liegt und was hinter uns liegt
ist nichts im Vergleich zu dem, was in uns liegt.

Henry David Thoreau – *Walden* oder Leben in den Wäldern

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Amtierender Dekan: Prof. Dr. Rhett Kempe

Prüfungsausschuss: Prof. Dr. Thomas Foken (Erstgutachter)
Prof. Dr. Andreas Held (Zweitgutachter)
Prof. Dr. Anke Jentsch (Vorsitz)
Prof. Dr. Christiane Werner Pinto

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Diploma theses supervised by myself:

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SUMMARY

In times of climate change and increasing carbon dioxide concentrations, three questions arise for ecosystem sciences: At first, which ecosystems can contribute to mitigate those processes? Secondly, how will ecosystems react on the changing conditions? And finally, is the performance of our commonly applied research methods adequate under those complex and continuously changing environmental conditions? This thesis is integrated in the joint research project FORKAST which investigates those questions. The role of grassland ecosystems' source or sink, related to the carbon cycle is currently not well-defined. At least, extensively managed grassland in mid European low mountain ranges may be able to contribute to climate change mitigation by carbon sequestration.

In ecosystem sciences, two dominant approaches are used to gain access to the carbon cycle. On the one hand these are the micrometeorological methods as the eddy-covariance technique which provides a top view from the atmosphere and, on the other hand, leading isotopic methods used in agricultural and soil science which allow a more interior view on the ecosystem. In this thesis, the advantages of both are turned to account.

In a first step, the investigated area, an extensively managed grassland in a mid European low mountain range, was defined as a net carbon sink. The carbon uptake accounted for $-91 \text{ g C m}^{-2} \text{ a}^{-1}$ in 2010. It has to be mentioned, too, that the long term climate measurements on the site revealed an upward trend of spring droughts. In a forty year time series a decrease of precipitation of 21 mm in April and May had been detected. Hence, the reaction of the carbon cycle was investigated by inducing a 1000-year spring drought event (i.e. 38 days without any precipitation) and comparing the carbon allocation into shoots, roots, soil and respiration fluxes to those detected on plots with normal precipitation. Therefore, a stable isotope pulse labeling experiment had been conducted. This fact indicated an increase of carbon allocation by 6.2% to below ground pools as soil and roots and a reduction of shoot respiration by 8.5% due to spring drought.

Gaining absolute values of carbon allocation, the relative portion, provided by pulse labeling and tracing, was set off the absolute carbon input into the ecosystem, obtained by eddy-covariance measurements of the net ecosystem carbon exchange in combination with partitioning of that into underlying assimilation and respiration flux. With the absolute

carbon input of $-7.1 \text{ g C m}^{-2} \text{ d}^{-1}$ and the relative allocation of the labeling, 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 could be determined and validated.

Flux partitioning is an important tool in ecosystem sciences. It can be accomplished in different ways. The commonly applied flux partitioning model based on Lloyd–Taylor and Michaelis–Menten functions had been compared to dark and transparent chamber measurements and to a partitioning by an isotopic approach, based on isoflux measurements with the relaxed eddy accumulation technique. The latter comparison revealed a lack of sensitivity of the common flux partitioning model for ecosystem reactions on short term changes in the weather conditions. The isotopic model based on detecting the isotope discrimination worked well on grassland compared to former experiments over a forest. Furthermore, relaxed eddy accumulation based $^{13}\text{CO}_2$ isoflux measurements confirmed only minor influences of atmospheric isofluxes on isotopic labeling experiments by detecting only a negligible portion of $^{13}\text{CO}_2$ of the entire CO_2 flux. However, there are certain restrictions for applying relaxed eddy accumulation on managed grassland, found in this study. Scalar similarity, a precondition for proper relaxed eddy accumulation fluxes, cannot be guaranteed directly after the management. It is suggested to wait at least 22 days in summer and 12 days in autumn after the management. The ecosystem needed this span of time to recover the regular source/sink distribution of water vapor, CO_2 and temperature.

The chamber method was applied to validate the assimilation flux, provided by the common flux partitioning model. This was done during the day at time of turbulent atmospheric conditions. In a comparison experiment between the chamber and eddy-covariance a good agreement was found at that time. In the late afternoon and during night, the chamber could not reproduce present atmospheric conditions, as, for example, increasing stable stratification due to the oasis effect or coherent structures. This resulted in smaller chamber CO_2 source fluxes of 26% during night and larger chamber CO_2 sink fluxes of 14% during day. The chamber technique is important for small scale measurements (especially in treatment experiments). Thus, it is important to know the reasons for those differences to eddy-covariance.

ZUSAMMENFASSUNG

Durch den Klimawandel und die steigenden Kohlendioxidkonzentrationen in der Atmosphäre stellen sich den Umweltwissenschaftlern drei entscheidende Fragen:

Welche Ökosysteme können einen Betrag zu Abschwächung dieser Prozesse leisten? Wie werden die unterschiedlichen Ökosysteme auf die veränderten Bedingungen reagieren? Und reichen die gegenwärtig angewendeten Untersuchungsmethoden aus um die komplexen und sich ständig verändernden Umweltbedingungen angemessen erfassen zu können?

Diese Arbeit befasst sich als ein Teil des Verbundprojektes FORKAST mit der Erforschung dieser Fragestellungen. Die Rolle von Wiesen als Kohlenstoffquelle oder -senke ist derzeit nicht klar definiert, wobei zumindest extensiv bewirtschafteten Wiesen in Mitteleuropäischen Mittelgebirgen eine Senkenfähigkeit zugesprochen wird.

Zwei Forschungsansätze prägen die Umweltwissenschaften in Bezug auf den Kohlenstoffkreislauf. Mikrometeorologische Methoden wie die Eddy Kovarianz Methode bieten eine Art Überblick aus der Atmosphäre, während sich die Boden- und Agrarwissenschaften über Isotopenanalysen eine Innenansicht des Ökosystems verschaffen. Die Vorzüge beider Forschungsfelder werden in dieser Arbeit gekoppelt.

Der erste Schritt war die eindeutige Definition des zu untersuchenden Extensivgrünlandes als Kohlenstoffsenke. Die Kohlenstoffaufnahme betrug im Untersuchungsjahr 2010 $91 \text{ g Kohlenstoff m}^{-2} \text{ a}^{-1}$. Zusätzlich wurde eine Zeitreihe über 40 Jahre mit Niederschlagsdaten ausgewertet, welche für das Untersuchungsgebiet eine steigende Tendenz zu Frühjahrs-trockenheit aufzeigten. In April und Mai wurde eine Gesamtabnahme der Niederschläge um 21 mm festgestellt.

Auf Grund dieser Erkenntnis sollte die Reaktion des Kohlenstoffkreislaufes untersucht werden. Eine 1000-jährige Frühjahrsdürre (d.h. 38 Tage ohne Niederschlag) wurde auf den Forschungsflächen künstlich erzeugt. Anschließend wurde die Kohlenstoffeinlagerung in Spross, Wurzeln, Boden und Spross- bzw. Bodenatmung auf diesen Flächen mit der unter normalen Niederschlagsbedingungen verglichen, wofür ein Markierungsexperiment mit dem stabilen Kohlenstoffisotop ^{13}C durchgeführt wurde. Die Frühjahrsdürrevariante zeigte

einen Anstieg der Kohlenstoffverlagerung in Wurzeln und Boden um 6.2 % und einen Rückgang der Sprossatmung um 8.5 %.

Um die Kohlenstoffverlagerung in Masseneinheiten angeben zu können, wurde die durch das Isotopenmarkierungsexperiment bestimmte relative Verlagerung mit der Masse an aufgenommenem Kohlenstoff verrechnet. Letztere betrug 7.1 g Kohlenstoff $\text{m}^{-2} \text{d}^{-1}$ und konnte über die Eddy Kovarianz Methode und eine modellbasierte Aufteilung des Netto-Ökosystemaustausches in seine Teilflüsse Assimilation und Respiration bestimmt werden. Es ergab sich ein Kohlenstoffeintrag von 2.5, 0.8, 0.5, 2.3 and 1.0 g Kohlenstoff $\text{m}^{-2} \text{d}^{-1}$ in Spross, Wurzeln, Boden, Sprossatmung und Bodenatmung.

Die Aufteilung des Netto-Ökosystemaustausches in die ihm zugrundeliegenden Flüsse ist in den Umweltwissenschaften von großer Bedeutung und kann auf unterschiedliche Weise bewerkstelligt werden. Die Ergebnisse des üblicherweise dafür angewendeten Modells nach Lloyd-Taylor und Michaelis-Menten wurden mit Messungen mit dunklen und transparenten Kammern und mit einem, auf Isotopenmessungen mit der Relaxed Eddy Accumulation Methode basierendem Modell, verglichen.

Das letztgenannte Vergleichsexperiment machte die fehlende Sensitivität des üblicherweise verwendeten Aufteilungsmodells für kurzfristige Wetterveränderungen und die entsprechenden Reaktionen des Ökosystems, deutlich. Das auf Isotopenmessungen basierende Modell berücksichtigt hingegen die Isotopendiskriminierung des Ökosystems und somit alle damit verbundenen Prozesse. Die Probleme bei der Anwendung, die auf Messflächen im Wald bekannt sind, traten auf dem Wiesenstandort nicht auf.

Zudem bestätigten die $^{13}\text{CO}_2$ Isoflussmessungen mit der Relaxed Eddy Accumulation Methode, dass der Anteil an $^{13}\text{CO}_2$ am Gesamtfluss und somit der Einfluss auf Isotopenmarkierungsexperimente vernachlässigbar ist.

Die Anwendung der Relaxed Eddy Accumulation Methode unterliegt jedoch auf extensiv bewirtschafteten Wiesenstandorten einer nicht zu unterschätzenden Einschränkung. Eine wichtige Voraussetzung für eine korrekte Flussbestimmung, die sogenannte Skalare Ähnlichkeit, ist kurz nach einem Grasschnitt nicht gegeben. Nach den Erkenntnissen dieser Studie ist eine Anwendung der Relaxed Eddy Accumulation Methode für 22 Tage im Sommer und für 12 Tage im Herbst nach einem Grasschnitt nicht empfehlenswert. Diese

Zeit hat das Ökosystem benötigt um die normalerweise vorliegende Quellen- und Senkenverteilung für Wasserdampf, CO₂ und Wärme wiederherzustellen.

Die Kammermethode wurde verwendet um den Assimilationsfluss aus dem Aufteilungsmodell zu überprüfen. Dieser Vergleich wurde während des Tages bei ausgeprägter atmosphärischer Turbulenz durchgeführt. Unter diesen Bedingungen wurde in einem Vergleichsexperiment zwischen der Eddy Kovarianz- und der Kammermethode die beste Übereinstimmung gefunden. Am späten Nachmittag und während der Nacht waren die Kammerergebnisse durch mangelhafte Abbildung der atmosphärischen Bedingungen verfälscht. Während der Nacht wurden durch Kohärente Strukturen hervorgerufene Flüsse nicht erfasst und am späten Nachmittag wurde die frühe, durch den Oaseneffekt hervorgerufene, Stabilisierung der bodennahen Luftschichten unterschätzt. In der Folge bestimmte die Kammer nachts einen um 26 % geringeren und tagsüber einen um 14 % höheren CO₂-Fluss. Für Messungen im kleinskaligen Bereich, zum Beispiel auf speziell behandelten Flächen, ist die Kammermethode nicht zu ersetzen. Deshalb ist es unerlässlich die Unterschiede zur Eddy Kovarianz Methode und deren Ursachen zu kennen.

1 INTRODUCTION

Climate change is real and its dependence on anthropogenic greenhouse gas emission is widely accepted in the scientific community. The increase of global carbon dioxide (CO₂) concentration induced ecosystem sciences to intensify their search for counter-measures and for identification of potential natural carbon sources and sinks. In this context, taking advantage of the natural carbon sequestration in ecosystems after carbon uptake by photosynthesis is one idea. Thereby, the importance of forests is more often emphasized in the discussion than of grassland ecosystems. That is because grassland ecosystems are not definitely defined as carbon source or sink. The IPCC ascribed the potential role of “source or sink” to grassland ecosystems (IPCC, 2013). Indeed, Janssens (2003) found a certain sink capacity which is of high uncertainty, though, and Ciais et al. (2010) as well as Gilmanov et al. (2010) could not find evidence either way. In contrast to that, there is a present sink potential for extensively managed grassland ecosystems in mid European low mountain ranges (Gilmanov et al., 2007; Allard et al., 2007; Ammann et al., 2007; Hussain et al., 2011). However, complex interactions between phenological development, management and atmospheric conditions create a certain inter-annual variability that can temporally turn the ecosystem into a carbon source (Wohlfahrt et al., 2008). Climate change enhances variations in the carbon cycle 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).

In this study, an extensively managed grassland site in a Bavarian low mountain range is to be defined as a carbon sink or source under present conditions. Long time climate data, collected at the measurement site in the city of Weißenstadt (620 a.s.l., 2.5 km northeast from the study site) confirmed altered precipitation patterns. A comparison of the 30 year climate period from 1961 to 1990 with the period from 1971 to 2000 pointed out a total decrease of precipitation by 21 mm in April and May (Foken, 2003). This is likely to induce drought stress already at the beginning of the vegetative period. Therefore, it requires further investigation – above all, with regard to potential consequences for the carbon cycle and the attributed sink potential of this extensively managed grassland in a mid European low mountain range. This issue fits well into the "Bavarian Climate Program 2020" of the Bavarian State Ministry of Sciences, Research and Arts, in cooperation with Bavarian universities, specialist government agencies and, to a great extent, with the research

cooperation BayFORKAST (Impact of Climate on Ecosystems and Climatic Adaptation Strategies), FORKAST (Bavarian State Ministry for Environment and Consumer Protection, 2013). Grasslands cover the surface of the Free State of Bavaria to a large percentage and - beneath the source/sink question, which is definitely important in terms of climate change mitigation – are of considerable importance for its economy. Consequently, the prospective value and the protection status, coming along with the resilience and the performance of the extensively managed grassland, have to be assessed on both counts.

Today, the carbon balance of a terrestrial ecosystem is commonly investigated by measuring the NEE using the eddy-covariance technique (e.g. Baldocchi et al., 2001; Aubinet et al., 2012). This direct method determines turbulent fluxes (Montgomery, 1948; Obukhov, 1951; Swinbank), requires certain correction and quality control tools (Foken and Wichura, 1996; Foken et al., 2004; Vickers and Mahrt, 1997) Mauder 2011 #191} and, for annual sums of NEE, gap filling mechanisms (Stoy et al., 2006; Ruppert et al., 2006a; Desai et al., 2008; Papale, 2012; Falge et al., 2001; Moffat et al., 2007). Those gap filling tools are closely related to flux partitioning models (FPM) which are designed for separating the NEE into its underlying components: ecosystem respiration (R_{ECO}) and gross primary production (GPP; Falge et al., 2002; Stoy et al., 2006; Desai et al., 2008; Lasslop et al., 2010; Rebmann et al., 2012). To parameterize temperature dependant R_{ECO} equal to nighttime NEE due to missing assimilation, the Lloyd–Taylor function was applied (Lloyd and Taylor, 1994). Light response regression on the basis of the Michaelis–Menten function (Michaelis and Menten, 1913) was used to parameterize daytime solar radiation dependant GPP. Those fluxes provide a better insight into the processes of the carbon cycle and have further advantages over the NEE as shown in the following. As the eddy-covariance method integrates the NEE over a large area of the meadow, the NEE is just the integrated result of all interacting processes that are related to the carbon cycle. Depending on whether the ecosystem is a carbon sink or a source in times of changing environmental conditions, it is important to know where carbon goes to or comes from (Gilmanov et al., 2007). At this point, atmospheric approaches reach their limits. Partitioning of assimilated carbon to various ecosystem pools can be achieved by using isotopic techniques (Buchmann, 2000, 2002; Kuzyakov, 2006). Thereby, natural continuous (C_3 plants grow after C_4 plants or vice versa), artificial continuous and artificial pulse labeling approaches have to be differentiated. Pulse labeling, being applicable the best and thus most conducted, tracer method (Kuzyakov and Domanski, 2000; Kuzyakov and Schneckenberger, 2004) is based on pulse-like insertion of a tracer to the green biomass – as the assimilating part of the ecosystem – and

subsequent sampling and tracing respectively in the diverse ecosystem compartments. Finally, it provides a relative proportion of the incorporated carbon translocated to various above and below ground carbon pools (Kuzyakov and Schneckenberger, 2004). However, the total amounts of translocated carbon remain unknown (Kuzyakov et al., 2001; Leake et al., 2006). This method is often used when sites with similar preconditions but different kinds of treatments are compared on the basis of ratios of carbon allocation (e.g. Hafner et al., 2012; Johnson et al., 2002; Allard et al., 2006). In this study it was initially applied to plots on extensively managed grassland where the already mentioned spring drought events were artificially induced and intensified before the labeled experiment. In comparison to the variant that was exposed to precipitation as usual, changes in the carbon allocation were detected for being able to assess the consequences when to find adequate climate change adaptation strategies (referring to the carbon cycle / the atmospheric CO₂ concentration).

The comparison of the atmospheric net ecosystem CO₂ fluxes of both variants (spring drought and regular precipitation) would constitute an upgrading of those experiments. At the study's relevant site, as lots of sites all over the world where the exchange of CO₂ between terrestrial ecosystems and the atmosphere is measured, the eddy-covariance technique is installed in place (Baldocchi et al., 2001). However, to measure small scale fluxes above a drought plot of 1 m², for example, is inappropriate because it integrates the signal over a large flux footprint (Rannik et al., 2012). Therefore, the application of a complementary technique often stands to reason: the chamber method. It has to be seen critically of course to investigate differently treated plots with different methods, at least as long as both methods are not compared properly. Former comparison studies between eddy-covariance and the chamber technique found differences, for example due to methodical problems under high vegetation (Subke and Tenhunen, 2004), at times with low turbulence intensity (van Gorsel et al., 2007), at night over complex surfaces (Myklebust et al., 2008), due to poor regression analysis in the chamber software (Kutzbach et al., 2007) or different target areas (Reth et al., 2005). Anyway, in most studies the lacking conformity during nighttime is mentioned. This circumstance suggests a more detailed investigation in times of stable stratification. There are also meteorological effects during daytime which would be worth investigating but still not considered in the scientific community. Closely related to the formation of atmospheric stability is the oasis effect that appears predominantly in the afternoon when a large upward latent heat flux causes cooling of the surface and thereby a downward sensible heat flux despite of a still incoming solar radiation (Stull, 1988; Foken, 2008). Firstly, it seems probable that this moisture dependent effect behaves differently on

drought plots and on those with regular soil moisture. Secondly, there may be effects on the carbon fluxes which may develop differently at a covered surface (under a chamber dome) and an undisturbed ecosystem. The same applies to atmospheric turbulence in general which simply cannot be reproduced within a chamber (Kimball and Lemon, 1971; Pumpanen et al., 2004; Rochette and Hutchinson, 2005). Atmospheric turbulence has a typical size spectrum and distribution of the turbulent eddies depending on height and surface structure. To a larger extent, low-frequency flow patterns, those are, coherent structures (Collineau and Brunet, 1993; Gao et al., 1989; Thomas and Foken, 2007), which are typical in the investigated region (Foken et al., 2012b), may cause differences between chamber and eddy-covariance measurement results. Consequently, for a proper comparison of both techniques it is necessary to compare not only daily sums of the NEE but also to take various atmospheric conditions in the course of the diurnal cycle into account. All these basic issues are to be clarified in a side by side measurement of the NEE, with latest chamber technology and the eddy-covariance technique with adequate quality criteria.

In contrast to chambers that – in combination with a darkened and transparent chamber – are able to determine ecosystem respiration and assimilation fluxes directly, eddy-covariance just provides the NEE as a combination of both. Then a more detailed information about the underlying fluxes is achieved usually by applying flux partitioning models (Stoy et al., 2006; Desai et al., 2008; Lasslop et al., 2010; Reichstein et al., 2012). However, those may provide fluxes with unrealistic temporal variation or magnitude (Stoy et al., 2006). An alternative in this context is partitioning, based on additional determination of the $^{13}\text{CO}_2$ isoflux (Yakir and Wang, 1996; Bowling et al., 2001; Knohl and Buchmann, 2005; Ogée et al., 2004; Wichura, 2009; Wichura et al., 2004; Ruppert, 2008; Lloyd et al., 1996). Due to physical and biochemical processes as stomatal uptake of CO_2 and photosynthesis, which discriminates against the heavier isotope ^{13}C , the air close to the biosphere gets enriched in $^{13}\text{CO}_2$ over the day. Consequently, the biomass itself and all following compartments within the dynamic carbon-cycle are depleted in ^{13}C and so is the respired CO_2 . Both effects account for a distinct diurnal cycle of the $^{13}\text{CO}_2$ concentration in ecosystem air (Flanagan et al., 1996; Lloyd et al., 1996). Resulting $^{13}\text{CO}_2$ isofluxes can be determined by hyperbolic relaxed eddy accumulation (HREA; (Bowling et al., 2001; Bowling et al., 2003; Wichura, 2009; Wichura et al., 2004) *inter alia*. Although laser supported $^{13}\text{CO}_2$ flux measurements get more and more important, REA technique is still applied today. Ruppert (2008) and Wichura (2009) investigated an isotopic approach by Lloyd et al. (1996) for partitioning NEE above forest ecosystems and found some restrictions due to complicated coupling conditions

(Thomas and Foken, 2007). This difficulty is avoided in the current study by applying the measurements for this approach tested on grassland. Thereby, the commonly used FPM for the NEE determined by eddy-covariance can be examined by a method that is based on a real ecosystem process, the discrimination of heavier isotopes by the biosphere. In addition to the general uncertainty about the carbon source/sink behavior of grassland mentioned in the beginning, the management of grassland ecosystems causes anomalies in the seasonal carbon cycle (Flechard et al., 2005). In the same way, temperature and water vapor fluxes, that is, sensible and latent heat fluxes may differ before and after the management and, additionally, this may come along with certain restrictions for REA measurements. Errors in the REA flux often appear when scalar of interest and proxy scalar behave differently in their turbulent transportation efficiency (Ruppert et al., 2006b). This so called scalar similarity is required especially for hyperbolic REA because two important factors – the hyperbolic deadband H and the proportionality factor b – and consequently the REA flux would be incorrect without (Oncley et al., 1993; Ruppert et al., 2006b). b -factors are often treated as constant (Meyers et al., 2006; Haapanala et al., 2006), although they underlie a certain diurnal variation. Other studies on managed ecosystems use CO_2 and water vapor (Baum and Ham, 2009) and mostly temperature (Myles et al., 2007; Hensen A. et al., 2009) as proxy scalar, sometimes shortly after the management (Nemitz et al., 2001). Thus, it has to be investigated thoroughly by numerous simulations with data from mown and unmown grassland, if this practice can be problematic and when REA experiments on managed grassland should not be conducted. Only with this information, correct $^{13}\text{CO}_2$ isofluxes can be ensured. Those fluxes serve another important purpose of this study, too. Pulse labeling experiments with $^{13}\text{CO}_2$ are applied all over the world in ecosystem research but the influence of atmospheric $^{13}\text{CO}_2$ fluxes was generally not considered (an overview is given by Kuzyakov and Domanski (2000) and Yakir and Sternberg (2000)). It has to be assessed if the influence on experiments based on isotope measurements can be neglected.

The overall motivation for all these technically ambitious experiments is to reduce present uncertainties about potential ecosystem reactions to contemporary changing environmental conditions. Furthermore, this is naturally based on research methods which are evaluated critically and in detail. Regarding this, four focal issues are revised in this study:

(a) The pulse labeling approach used in plant and soil sciences requires an upgrade. Up to now, an essential constraint of the method is to gain relative portions of translocated carbon only. However, most studies related to carbon balance and turnover mass units are important

(Kuzyakov and Domanski, 2000). With a thoroughly evaluated carbon input flux to the ecosystem this constraint can be reserved. For this purpose, a completely novel approach was conceived by looking for a steady state of the relative carbon distribution to the different carbon pools after the pulse labeling (cf. Saggarr et al., 1997; Saggarr and Hedley, 2001; Wu et al., 2010) and by setting this result off against the average absolute carbon input to the ecosystem during the period between labeling and steady state, determined by eddy-covariance in combination with a flux partitioning model. This first-time performed combination of methods can be seminal in a more and more integrated field of atmosphere, plant and soil sciences in times of presently changing environmental conditions (Appendix A).

(b) From an atmospheric point of view an upgrade is required, as well. Eddy-covariance measurements are well established and its quality profits from sophisticated quality assessment tools but it also suffers from two constraints which are worth noting. For a better insight to the carbon cycle, NEE needs to be partitioned into its source and sink fluxes by flux partitioning tools. Consequently, those have to be evaluated by a method that is based on a real ecosystem process, the discrimination of heavier isotopes by the biosphere (Ruppert, 2008). Therefore, REA represents a decent technique (Appendix B).

(c) Moreover, eddy-covariance is an atmospheric measurement technique and is installed in a certain height above the ground. Thus, it measures a large flux footprint (Rannik et al., 2012) and is unsuitable for a determination of fluxes above small, in experiments often treated, plots, as for instance the drought plots in this study. There, the chamber method and adequate comparison to eddy-covariance under consideration of the atmospheric conditions become relevant (Appendix C).

(d) In respect of climate change the extensively managed grassland was to define as carbon source of sink by determination of the annual carbon balance with the eddy-covariance technique and the influence of spring drought events on the carbon-cycle were to define by stable isotope pulse labeling (Bavarian State Ministry for Environment and Consumer Protection, 2013).

2 METHODS AND EXPERIMENTS

In the first part of this chapter the most important scientific methods applied in this study are introduced. The second part describes how these methods interacted within the respective field experiments.

2.1 Methods

2.1.1 Eddy-covariance

The eddy-covariance technique is, by definition, a direct method (Montgomery, 1948; Obukhov, 1951; Swinbank) for measuring turbulent fluxes. For the determination of the CO₂ flux, the concentration was measured by an open-path gas analyzer (LI-7500, LI-COR Biosciences, Lincoln, Nebraska USA) and the wind vector by a 3D sonic anemometer (CSAT3, Campbell Scientific, Inc., Logan, UT USA) at high frequency (20 Hz), 2.5 m above ground. Data had been stored on a data logger (CR3000, Campbell Scientific, Inc., Logan, UT USA) and collected daily by a computer system as a backup. Data had also been post processed and quality controlled, based on latest micrometeorological standards by the software package TK2 which had been developed at the University of Bayreuth (Mauder and Foken, 2004). In the meantime, this still evolving software (TK3) has become available: Mauder and Foken (2011) incorporates all necessary data correction and data quality tools (Foken et al., 2012a). It was proved successfully in comparison to six other commonly used software packages (Mauder et al., 2008). For every averaging interval of 30 minutes, the included state of the art quality flagging system evaluated stationarity and turbulence and marked the resulting flux with quality flags from 1 (very good quality) to 9 (very low quality; Foken and Wichura, 1996; Vickers and Mahrt, 1997; Foken et al., 2004). Only data with quality 3 or better has been used in this study. Also footprint analysis after Göckede et al. (2004), Göckede et al. (2006), Rannik et al. (2000) and (2012) was performed to assure that the measured data represented exclusively the land use type of interest: extensively managed grassland.

2.1.2 Relaxed eddy accumulation

The basic idea of Desjardins in 1972 (Desjardins, 1977) of separating the vertical wind into an up- and downward component was applied by Businger and Oncley (1990). This Eddy

Accumulation method (EA) was combined with the flux–variance–similarity to create an indirect method: the relaxed eddy accumulation (REA). The REA–flux

$$F_{REA} = b \sigma_w \rho_a (\overline{c_\uparrow} - \overline{c_\downarrow}) \quad (1)$$

is derived from average up- and downward scalar concentration $\overline{c_\uparrow}$ and $\overline{c_\downarrow}$, standard deviation of the average vertical wind velocity σ_w , density of dry air ρ_a and an empirical, dimensionless proportionality factor b that compensates for the loss of information due to the mentioned “relaxation” (Ruppert et al., 2006b). To reduce relative errors in flux determination individually simulated b –values for every measurement location and period have to be favored over application of a constant b (Ruppert et al., 2006b; Foken, 2008). Thus, b is determined from a proxy scalar, a second scalar quantity which can be measured with high temporal resolution (by eddy-covariance) and which behaves similarly in atmospheric transport (Ruppert et al., 2006b; Ruppert et al., 2012). This is described in the theory of scalar similarity (Kaimal et al., 1972; Pearson et al., 1998). The proportion of both proxy scalar fluxes, F_{REA} and the eddy-covariance flux ($F_{EC} = \overline{w'c'}$), provides b :

$$b = \frac{\overline{w'c'}}{\rho_a \sigma_w (\overline{c_\uparrow} - \overline{c_\downarrow})} \quad (2)$$

However, not until Businger and Oncley (1990) had modified the method by discarding fluctuations around zero – that has only a small influence on the entire flux, anyhow – by introducing a deadband, could the REA idea be implemented with regard to mechanical restrictions of the speed of valve switching.

$$b(w_0) = \frac{\overline{w'c'}}{\rho_a \sigma_w (\overline{c_\uparrow}(w > w_0) - \overline{c_\downarrow}(w < -w_0))} \quad (3)$$

The size of the linear deadband w_0 around zero is determined individually according to the experimental conditions and the particular scalar of interest. The same applies to $b(w_0)$ which has to be determined individually by associated simulations with proxy scalars.

To maximize scalar concentration difference between up- and downdraft air samples, an application of a hyperbolic deadband H is recommended (Bowling et al., 1999b) and which is required, for example, for differences in the order of the measurement precision. By application of H in hyperbolic REA (HREA), $b(H)$ is reduced to lower values around 0.22 ± 0.05 (Bowling et al., 1999b), 0.15 – 0.27 (Ruppert et al., 2006b), respectively. H is

based on the fluctuation of the vertical wind velocity w' and the concentration c' of a proxy scalar as well as their standard deviations $\sigma_{w/c}$:

$$\left| \frac{w' c'}{\sigma_w \sigma_c} \right| \geq H \quad (4)$$

Adequate scalar similarity is required especially for hyperbolic REA because H depends on the proxy scalar concentration. Generally, $b(H)$ and the REA flux as the final product will be flawed without scalar similarity between the scalar of interest and the proxy scalar (Onclay et al., 1993; Ruppert et al., 2006b). Differences in distribution (Andreas et al., 1998a; Ruppert et al., 2006b; Held et al., 2008), amount, and strength (Katul et al., 1999; Katul and Hsieh, 1999) of scalar sources and sinks are reasons for differences in turbulent exchange of the scalars and in scalar similarity. Due to the fact that scalar similarity is influenced by the seasonal variation of canopy physiology (Williams et al., 2007), the effect of the management events on the scalar similarity between CO_2 and the sonic temperature and water vapor had been analyzed in this study by calculating correlation coefficients r (c.f. Gao, 1995; Katul and Hsieh, 1999; Ruppert et al., 2006b; Held et al., 2008):

$$r_{c_{proxy1}, c_{proxy2}} = \frac{c'_{proxy1} c'_{proxy2}}{\sigma_{c_{proxy1}} \sigma_{c_{proxy2}}} \quad (5)$$

For more detailed information see Appendix B.

2.1.3 Stable isotope pulse labeling

Pulse labeling with the stable carbon isotope ^{13}C provides information about the relative allocation of recently assimilated carbon to different carbon pools (Ratray et al., 1995). Therefore, the assimilating plant parts are exposed to the $^{13}\text{CO}_2$ isotope-tracer in a transparent chamber for a short period of time (three hours). The chamber has to be sealed and, furthermore, cooling and turbulent mixing of the chamber air has to be guaranteed (Paterson et al., 2009). For more detailed information about the chamber construction see Drösler (2005) and Appendix A. Translocation of the assimilated ^{13}C had been 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, dried, weighted and milled. Also unlabeled natural abundance samples were taken and treated in the same way. For the final determination of the relative carbon input into the different compartments, several calculation steps were necessary. The enrichment of ^{13}C in a certain carbon pool was derived

from subtracting the naturally abundant amount from the amount of ^{13}C in the labeled pool. Then, the total amount of ^{13}C in the pool was calculated by multiplication with the amount of total carbon in the pool. Total amounts of ^{13}C were found immediately after the labeling was summed up over all investigated pools as a reference value for the recovered amount of ^{13}C during the subsequent sampling period. Processing in this way, the ^{13}C amounts of every single pool at every point of time could be related to this total value and, additionally, the recovery of the tracer and thereby the relative proportion of the carbon allocation could be determined (cf. Hafner et al., 2012 and Appendix A).

2.1.4 Flux partitioning

In order to gain finally the absolute carbon input into the ecosystem from the NEE measured by eddy-covariance, two tasks were performed: Due to rejection of outliers and low quality data, gaps occurred within the 30-minute 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, Lloyd–Taylor function had been 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., 2006a). 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).

Applying transparent and dark soil chambers in combination represents an alternative partitioning method. By a simple subtraction, GPP can be determined from measured NEE and R_{ECO} .

Beside the REA technique and the determination of the $^{13}\text{CO}_2$ isoflux (F_{ISO} ; e.g. Yakir and Wang, 1996; Bowling et al., 2001; Ruppert, 2008), a third approach has to be mentioned. Therefore, the isotope ratios of assimilated (δ_A) and respired CO_2 (δ_R) are determined with the REA device (Ruppert, 2008) and by analyzing soil respiration samples on the basis of the Keeling plot method (Keeling, 1958). Finally, F_{EC} can be partitioned into assimilation

$$F_A = \frac{F_{\text{ISO}} - \delta_R F_{\text{EC}}}{\delta_A - \delta_R} \quad (6)$$

and respiration flux

$$F_R = \frac{F_{ISO} - \delta_A F_{EC}}{\delta_R - \delta_A} \quad (7)$$

in accordance to Lloyd et al. (1996), Bowling et al. (2001), Bowling et al. (2003), Ruppert (2008) and Wichura (2009). For more detailed information please see Appendix B.

2.1.5 Chamber system

The applied system (LI-8100-104C, transparent for NEE measurements at low vegetation, LI-COR Biosciences, Lincoln, Nebraska USA) was an automated flow-through non-steady-state soil chamber where sample air was constantly held circulating between the chamber and an infrared gas analyzer (IRGA) by a rotary pump with 1.5 L min^{-1} through a chamber volume of 4822 cm^3 . The CO_2 flux was estimated by the rate of CO_2 concentration change inside the chamber during a 90 second close time. The chamber had a lift-and-rotate drive mechanism which rotated the bowl-shaped chamber 180° away from the collar. This shape allowed a good mixing by means of the circulation of the sample air through the IRGA alone, without a ventilator (LI-COR, 2004). Barometric and, above all, turbulence-induced pressure fluctuations above ground surface level influence the efflux from the soil. Thus, modern chambers are equipped with a venting tube that transmits atmospheric pressure changes to the chamber headspace (Rochette and Hutchinson, 2005). LI-COR had installed a patent-pending pressure vent with tapered cross section at the top of the chamber that minimizes pressure pulses at chamber closing and allows the tracking of ambient pressure under calm and windy conditions by eliminating the Venturi effect (Conen and Smith, 1998) occurring at former simple open vent tubes (Xu et al., 2006). The exchange through the venting tube is negligible compared to the CO_2 diluting effect by water vapor during the measurement which in turn is corrected by the measurement software (LI-COR, 2004). NEE is measured by a chamber with a transparent dome enabling assimilation as well as respiration processes inside. The transparent chamber for the NEE comparison was closed for 90 seconds four times during a half-hour period. In the meantime, the system was flushed for 135 seconds and the dark chamber for R_{ECO} was closed for 90 seconds. The system was then flushed with ambient air again. The closing and opening process of the transparent chamber as part of the flushing time lasted 13 seconds each.

2.2 Experiments

2.2.1 Study area

All experiments of this study were conducted on the extensively cultivated submontane grassland site “Voitsumra” at the edge of the low mountain range “Fichtelgebirge” in northeast Bavaria, Germany, located 624 m a.s.l. (50°05′25″N, 11°51′25″E). For the last 10 years, the site had been 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) 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.) is situated north of the study site and the “Schneeberg” (1051 m a.s.l.) is south of it. Together, these two mountains generate a channeled wind field for the site with East and, especially, West as the dominating wind directions (prevailing wind direction 263°). Thus, disturbances of the turbulence measurements could easily be avoided by installing all other experimental devices close to the eddy-covariance mast but perpendicular to the main wind direction. The plant community is described as *Molinio–Arrhenatheretea* R. Tx. 1937 – economic grassland and the most dominant of the 48 species are *Alchemilla monticola*, *Juncus filiformis*, *Polygonum bistorta*, *Ranunculus acris* and *Trifolium repens*.

All experiments described in the following had been supported by an automated weather station which provided 10 minute averages of a range of climate data as up- and downwelling short- and long-wave radiation, air and soil temperature, humidity and soil moisture and precipitation. Those data were necessary for quality assessment and as input parameters for flux partitioning.

2.2.2 Labeling and drought experiment

The pulse labeling experiment was conducted during summer 2010 from 16 June to 6 July for two different reasons. Firstly, to apply the novel coupling approach with the eddy-covariance technique and, secondly, to evaluate potential influences of a 1000-year spring drought event on the carbon cycle. Therefore, the manipulation strength could be estimated by fitting the Gumbel distribution (Gumbel, 1958), a generalized extreme value distribution (GEV), to a climate data series measured at the area of research (Jentsch et al., 2007). This circumstance resulted in an artificial spring drought, beginning on 9 May 2010 and lasting

38 days. It was induced by covering five of the ten plots of the labeling experiment with transparent and well-aerated rain-out shelters. The stainless steel soil frames for attaching the labeling chambers were pre-installed three weeks before the actual labeling experiment, too, to keep disturbances of plants and soil as small as possible. Soil moisture was monitored with TDR devices on both, drought and regular, variants. Hereafter, the ^{13}C pulse labeling and a 21 day tracing period was conducted to gain the relative carbon distribution under regular and drought conditions finally. Accompanying eddy-covariance measurements – conducted for the whole year 2010 for the annual carbon balance – the carbon net ecosystem provided exchange on the site. In combination with the flux partitioning model (see. 2.1.4), the absolute carbon assimilation could be set off against the relative carbon distribution found at the regular plots. However, on the small scale drought plots fluxes can not be determined by eddy-covariance technique. Therefore, the chamber method is suggested.

2.2.3 Chamber – eddy-covariance comparison

Due to the relevance of the chamber method for small scale flux measurements and the necessity to appraise frequently the comparability of the chamber method and eddy-covariance, NEE, measured with both techniques, had been compared in an experiment from 25 May to 3 June in 2011. Turbulent flux data were taken from the long term carbon balance measurements on the site. The size of the fluxes is closely connected to the diurnal cycle. To be able to investigate differences between both methods and underlying short-term effects, the difference between eddy-covariance and chamber flux was normalized with the eddy-covariance flux.

For the chamber measurements soil collars which included an area of 318 cm^2 were pre-installed 10 cm deep in the soil two weeks before the experiment to create a perfect seal and to avoid disturbances of the CO_2 efflux by cut and wounded plant roots at the beginning of the measurement period. Due to the channeled wind field on the site (2.2.1), the chamber could be installed very closely to the eddy-covariance mast without disturbing the flux footprint.

2.2.4 Relaxed eddy accumulation experiments

Relaxed eddy accumulation (REA) simulations serve to parameterize REA measurements but also to evaluate present conditions on the site for REA application. The simulations were accomplished in the periods before and after mowing and aftermath events on the extensively managed grassland site in September 2009, July 2010, September 2010 and August 2011. During the whole 16 days before and the 27 days after management, similar atmospheric conditions were investigated focusing on potential effects of the management on scalar similarity and the determination of the proportionality factor b .

The real REA field experiments were accomplished during the main growth period of 2010 on 22 June and 25 August with an adequate time lag to the last management event. The REA device used in this study (Fig. 1) was constructed and tested by (Ruppert et al., 2012) and has already been applied in other field experiments (Ruppert, 2008). REA measurements require high frequency vertical wind velocity and CO₂ concentration data provided by the ultra-sonic anemometer and the infra-red gas analyzer of the eddy-covariance mast at the site. That one also provided the NEE flux for the comparison of Lloyd–Taylor / Michaelis–Menten and REA-isoflux based flux partitioning (2.1.4). Isofluxes (F_{ISO}) can be derived from CO₂ concentration ($\overline{C_{\uparrow\downarrow}}$) and $\delta^{13}\text{C}$ -isotope ratio ($\overline{\delta^{13}\text{C}_{\uparrow\downarrow}}$) differences in up- and downdrafts (Bowling et al., 1999a; Ruppert, 2008; Wichura, 2009):

$$F_{ISO} = b(H_{eff}) \sigma_w \rho_a \left(\overline{\delta^{13}\text{C}_{\uparrow} C_{\uparrow}} - \overline{\delta^{13}\text{C}_{\downarrow} C_{\downarrow}} \right) \quad (8)$$

ρ_a is the density of dry air, σ_w derived from the time series of the vertical wind velocity measured by the ultra sonic anemometer and the proportionality factor b , in this case, was determined from the effectively measured CO₂ fluxes measured by REA and eddy-covariance during the experiment.

F_{ISO} is also interesting to evaluate the influence of atmospheric isotope fluxes on experiments based on isotope measurements, for example after an isotopic labeling.

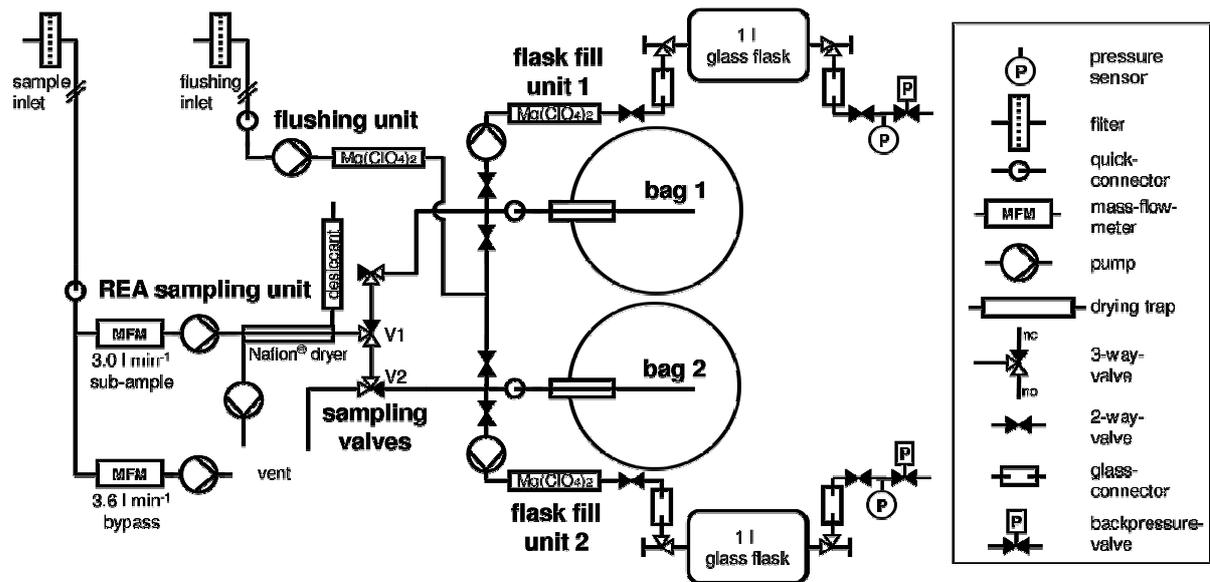


Figure 1: Design of the REA device (cf. Ruppert et al., 2012)

3 RESULTS

3.1 Annual carbon balance

The NEE was directly measured by eddy-covariance in 2010 (Fig. 2) and resulted for $-249 \text{ g C m}^{-2} \text{ a}^{-1}$. After subtraction of the harvest output of $158 \text{ g C m}^{-2} \text{ a}^{-1}$, $-91 \text{ g C m}^{-2} \text{ a}^{-1}$ still remained (Fig. 3), identifying the site as relatively big carbon sink in relation to other comparable extensively managed grassland (Table 2 in Appendix A).

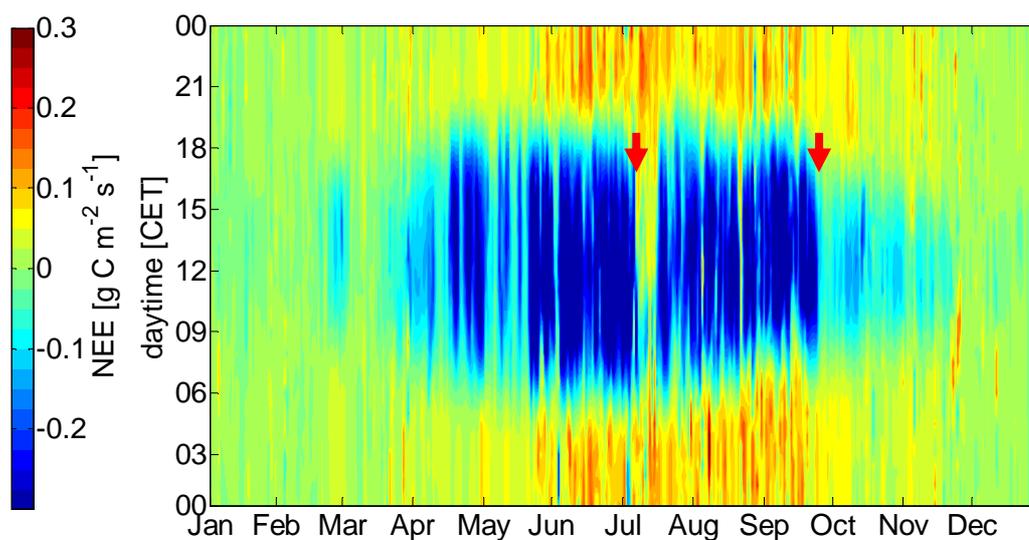


Figure 2: Net ecosystem carbon exchange (NEE) in 2010 with half-hourly resolution; mowing and aftermath – labeled with red arrows – are clearly visible.

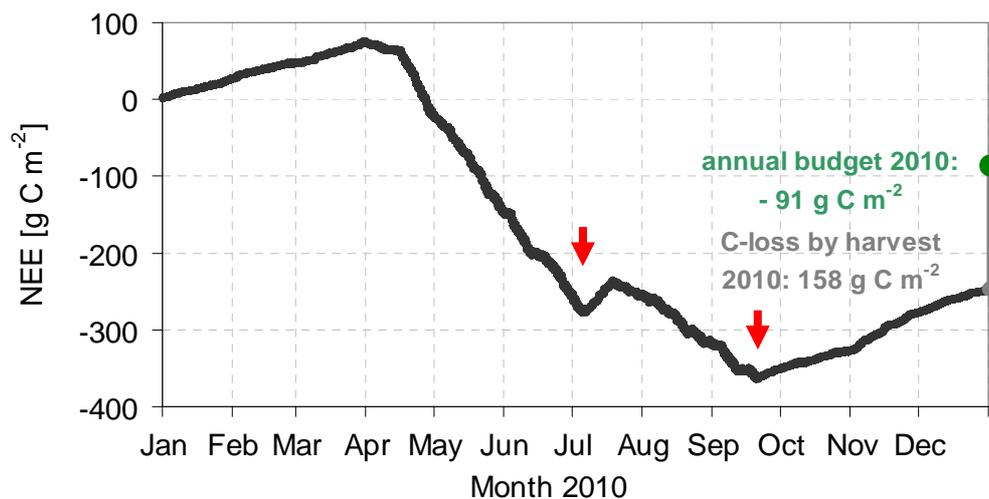


Figure 3: Annual carbon budget and carbon loss by harvest in 2010; red arrows show mowing and aftermath

The existing sink capacity implicates the ecosystem to be worth further investigating if this potential can be upheld or maybe degrade in times of climate change. Therefore, the reaction on present spring drought events had to be analyzed.

3.2 Effects of spring drought on carbon allocation

38 days before the pulse labeling experiment started, five of the ten plots had been covered with rain-out shelters to simulate a 1000-year spring drought event. The 21-day-period of sampling and tracing began immediately after the $^{13}\text{CO}_2$ pulse labeling on 16 June 2010. In doing so, differences in the ^{13}C recovery rate during the tracer translocation period could be detected. From the first sampling onwards, the ^{13}C recovery in the shoot biomass decreased in both variants (Fig. 4a and c). During the first day after the labeling, the loss of ^{13}C by shoot respiration may be the most important reason for this as the steep increase of the ^{13}C recovery in the regular variant proves (Fig. 4a). However, this increase is less pronounced in the drought variant (Fig. 4c). Here, the first differences become obvious, probably caused by reduced stomatal conductance coming along with drought stress. The maximum ^{13}C amount attained by the dry and normal soil pool was not detected until one day after the labeling. In case of the root ^{13}C dynamics, merely tendencies can be discussed because of the large variations. But, obviously, there is a larger input into the root pool in the drought version in sampling day 3 and 4 (Fig. 4a and c). Similar to shoot respiration, the increase of the ^{13}C loss by soil CO_2 efflux has its maximum during the first day and then abates over time. At the end of the experiment all values are stabilizing and there are no more significant changes than in at least the last two samplings of each of the five pools in both variants.

The final percentages at the end of the translocation process are illustrated in Figure 4b and d. Although there were no significant differences in the investigated carbon pools between the two variants, an increase of 6.2% stands for a tendency to higher carbon allocation to below ground pools as soil and roots during spring drought (Fig. 4b and d). This comes along with a reduced shoot respiration (-8.5%).

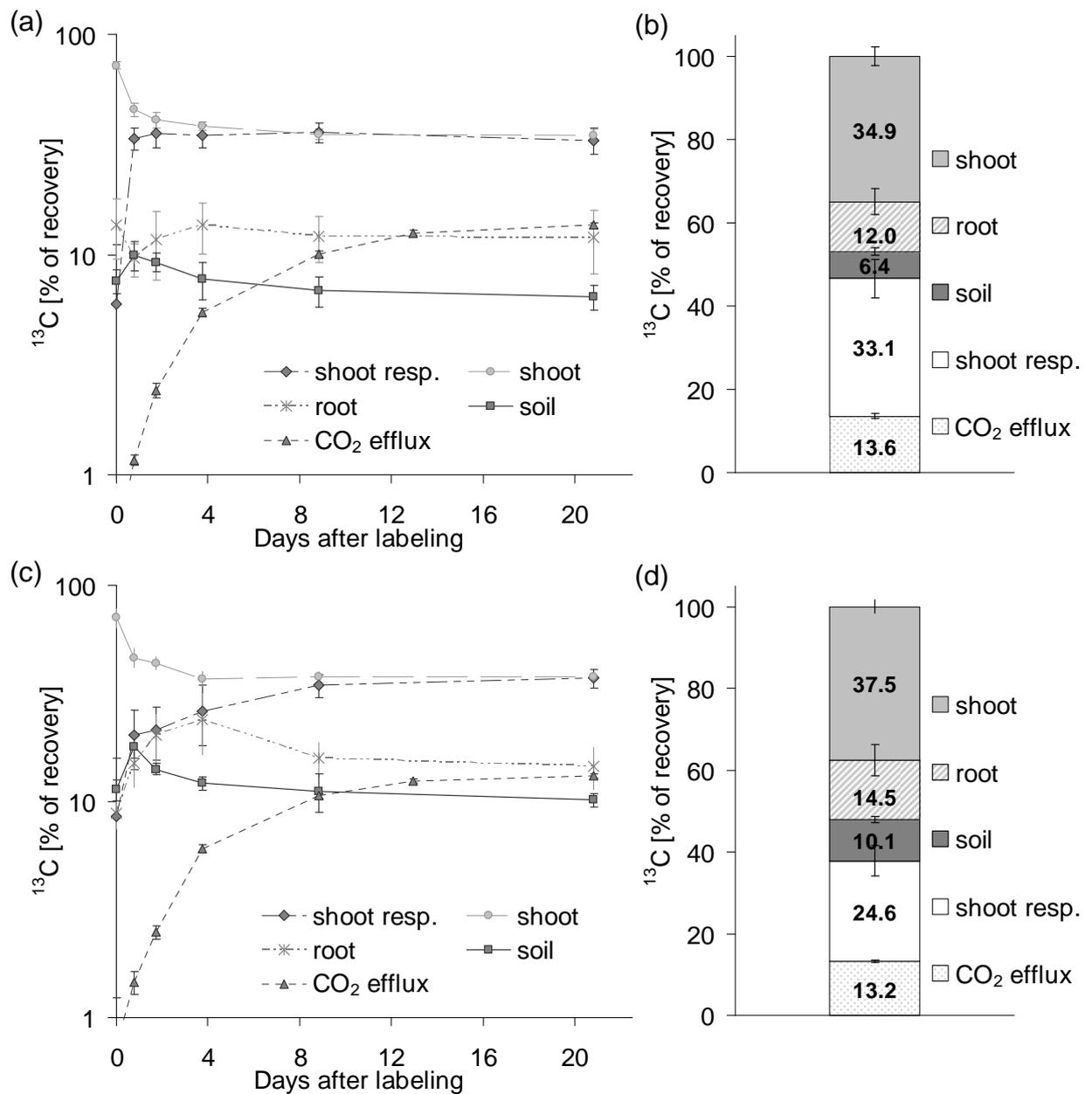


Figure 4: ^{13}C tracer dynamics during the chase period in all carbon pools under regular precipitation conditions (a) and after 38 days of spring drought (c); (b) and (d) illustrate relative proportions of ^{13}C recovered, that is, final distribution by percentage at the last day of sampling (day 21 of the chase period) in respective ecosystem carbon pools; The x-axis of (a) and (c) intersects at $y = 1\%$ for a better illustration. Only one value remains below 1% each which is the $^{13}\text{CO}_2$ efflux immediately after the labeling accounting for 0.5% (regular) and 0.7% (drought); Error bars represent standard errors of the mean ($\pm\text{SEM}$).

For further investigation, the absolute instead of the relative carbon allocation would be required. On the small drought plots, eddy-covariance measurements are not possible. The chamber method could be an adequate substitute for that. However, uncertainties of those measurements due to insufficient reproduction of the atmospheric conditions are well known (Rochette and Hutchinson, 2005). Thus, eddy-covariance – chamber differences had been evaluated in detail and the results are presented in the following.

3.3 Chamber – eddy-covariance comparison

Already the first impression of the data of the ten day eddy-covariance – chamber side by side NEE measurement suggested a further and more detailed analysis. Smaller chamber CO₂ source fluxes of 26% during the night and larger chamber CO₂ sink fluxes of 14% during the day (negative sign) resulted in an absolute value of the chamber sink flux that was 40% larger than that which was measured by eddy-covariance. This is similar to other studies (Wang et al., 2009; Fox et al., 2008). A first indication as to the cause of the large difference at night may be provided by the kind and dimension of scattering of the measured fluxes. While daytime CO₂ flux results of both techniques scatter quite similarly, nighttime chamber fluxes scatter less than half as much as the eddy-covariance fluxes: the chamber measures a virtually constant flux during the night (cf. Janssens et al., 2001). For this kind of aggregation of the positive chamber fluxes (cf. Laine et al., 2006), distinctly associated reasons could be detected, predominantly at times with high atmospheric stability (Fig. 5b), low wind velocity (Fig. 5c) and a cool ground surface, that is, little outgoing long-wave radiation (Fig. 5d). While the eddy-covariance system responds to the smallest changes of the atmospheric conditions as well as the nighttime ecosystem respiration flux does, the chamber is directly connected to the ground surface – where the ecosystem respiration is more or less constant – with only minor influences from the surrounding atmosphere (Lai et al., 2012; Norman et al., 1997; Reth et al., 2005), transferred into the chamber system exclusively by the pressure vent (Xu et al., 2006). While eddy-covariance measures that wide range of CO₂ fluxes, the parameters illustrated in Figure 5b, c and d turned out to be responsible particularly for the uniformity of the chamber flux.

During night, an increasing exchange by coherent structures was detected, as well. Coherent structures are generated by braking gravity waves or under the influence of low level jets (Karipot A. et al., 2008) and can cause 50–100% of the gas exchange during nighttime and

10–20% during day above a forest (Thomas and Foken, 2007). The influence of coherent structures might be less above meadows due to the negligible mixing layer (roughness sublayer). It was found out that some of the highest eddy-covariance fluxes in times of uniform chamber performance occurred together with large coherent structure fluxes (Fig. 6b). In the experiment region, coherent motions had already been detected as a consequence of low-level jets reaching the ground and breaking gravity waves (Foken et al., 2012b). Coherent structures appear sporadically (average in this study: 38 h^{-1}). Thus, the total size of the coherent structure flux is less than the typical turbulent flux, yet coherent motions produce a turbulence which is obviously recognized by eddy-covariance but not by the chamber technique. Coherent structures, as well as heating due to dewfall, cause slightly higher turbulent fluxes during nighttimes. The condensation heat, thereby, reduces the downward sensible heat flux and the strong stable stratification. Both processes are related to slightly higher wind velocities and to larger eddy-covariance flux results. Eddy-covariance and chamber which measured nighttime respiration fluxes at high wind velocities (largest 25%, $u > 2.9 \text{ ms}^{-1}$) are within the same range close to the bisecting line in Figure 6a but with a significant tendency to larger eddy-covariance fluxes. That fact coincides with a study of Denmead and Reicosky (2003) who found an increase of the eddy-covariance – to chamber–flux ratio with the wind velocity. Although the chamber reproduces the flux variations very well at high wind velocities, this means that it has the ability to describe small as well as larger fluxes, it generally underestimates the flux. Hence, at night, in addition to the stratification effect, situations with high wind velocities result in larger eddy-covariance than chamber CO_2 fluxes.

In the late afternoon, CO_2 sink fluxes, which had been measured by the chamber, prevail. Those were sustained larger and longer into the evening, resulting in a flux up to twice as large as the eddy-covariance flux. The reason was defined as the oasis effect, which is named after the moisture-dependent cooling effect occurring in oases and which is defined as the sensible heat flux (Q_H) changing to negative values in combination with a still large positive latent heat flux (Q_E) and solar radiation (Stull, 1988; Foken, 2008). In Figure 5a, nearly all measurements which are influenced by the oasis effect show larger chamber fluxes. Also two thirds of the situations with contrary eddy-covariance–chamber flux directions (filled circles in Figure 5a) and the higher sink fluxes of the chamber at small values could be explained directly by the oasis effect (large black circles in Fig 5a). Chamber fluxes are larger because in the chamber the long wave radiation balance is altered to almost zero. However, there is a physical barrier to the surrounding, increasingly stable

stratified air masses. This evokes reduced surface cooling, weak development of stable stratification and finally higher fluxes compared to eddy-covariance. With the sunset, the remaining assimilation potential is gone and the difference between both systems declines.

Since the oasis effect is relevant for daytime chamber flux measurements, it is important to be considered for assimilation flux measurements with the chamber method, for example for partitioning issues. Such efforts should better be accomplished from late morning – when all instruments have dried from dewfall – until afternoon when the oasis effect gains more and more influence.

Moreover, there are two other methods for partitioning of the NEE based on Lloyd–Taylor and Michaelis–Menten functions and on isoflux measurements with the relaxed eddy accumulation technique.

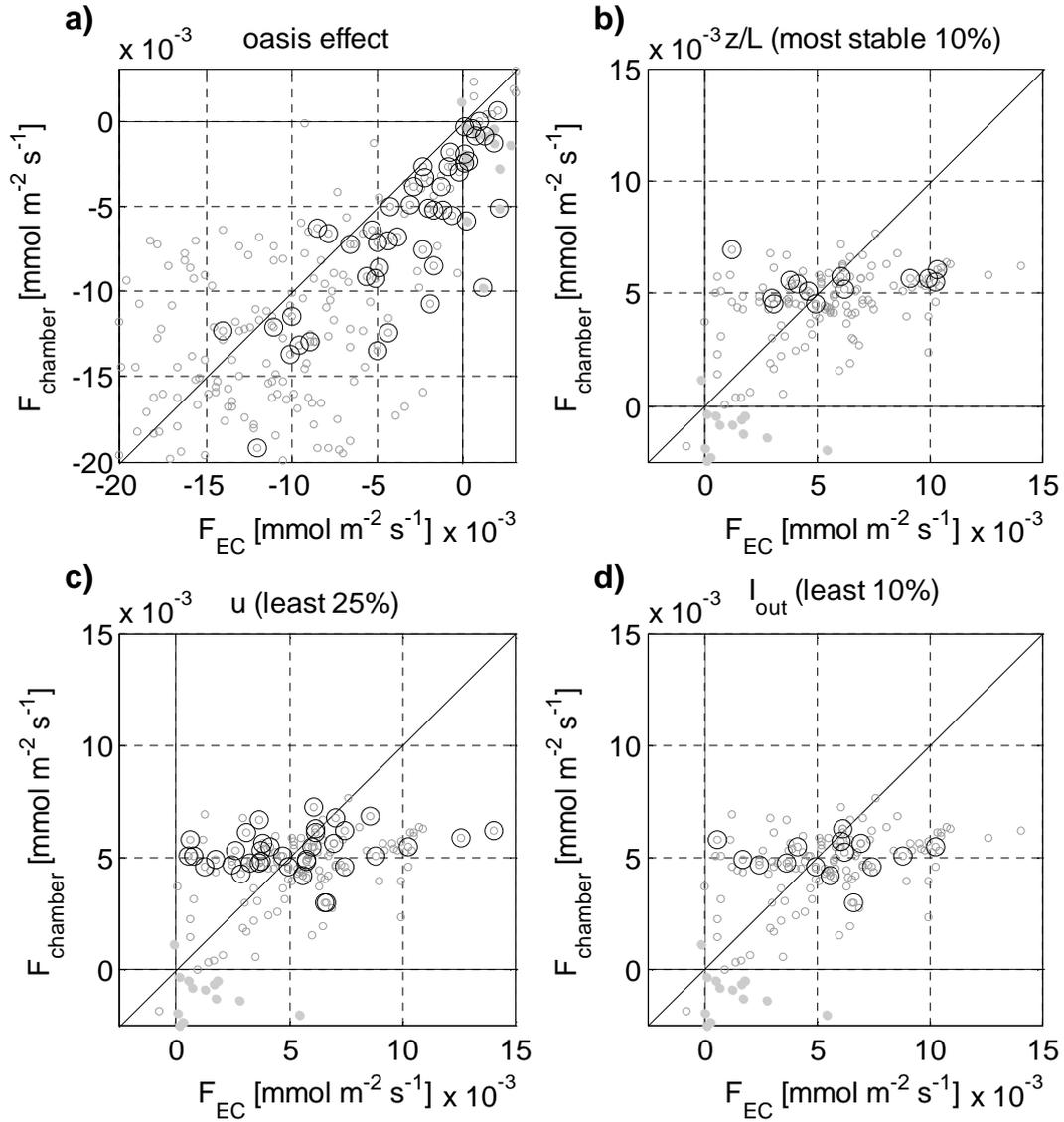


Figure 5. Scatter plot sections of eddy-covariance– and chamber–determined-NEE under particular micrometeorological conditions: a) oasis effect; b) atmospheric stability $z/L > 0.7$; c) wind velocity $u < 0.9$ m s⁻¹; d) outgoing long wave radiation $I_{\text{out}} < 319$ Wm⁻² – labeled with large black circles in each case; light grey circles represent fluxes with different directions.

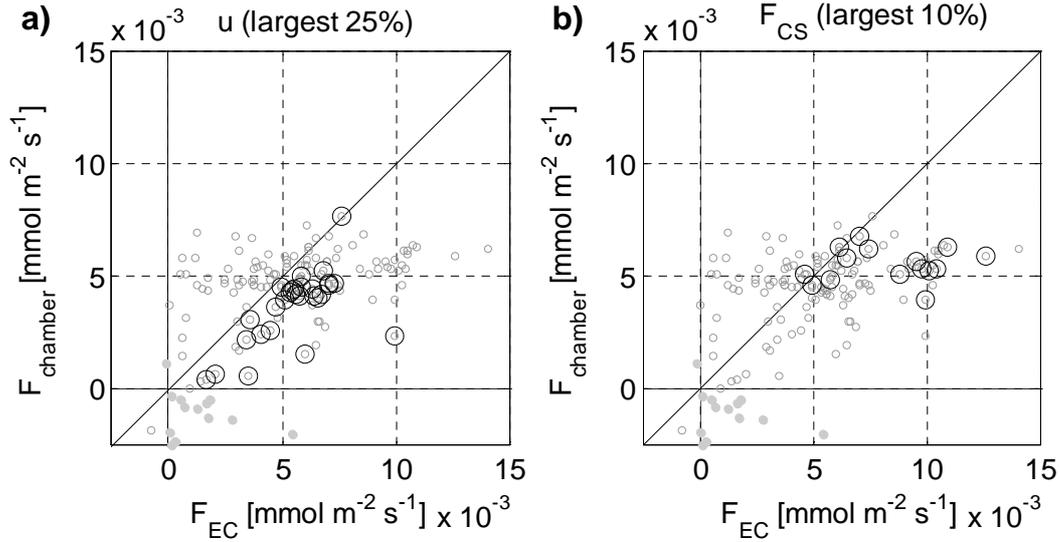


Figure 6. Scatter plot sections of eddy-covariance– and chamber–determined NEE under particular micrometeorological conditions: a) largest 25% of the wind velocities ($u > 2.9 \text{ ms}^{-1}$); b) largest 10% of the fluxes due to coherent structures ($F_{CS} > 0.0015 \text{ mmol m}^{-2} \text{ s}^{-1}$) – labeled with large black circles in each case, light grey circles represent fluxes with different directions.

3.4 REA application and flux partitioning

Application of relaxed eddy accumulation (REA) on managed grassland requires a detailed evaluation of scalar similarity, for being the first precondition for proper determination of the proportionality factor b and consequently the REA-flux. In this thesis, scalar similarity was detected by calculating correlation coefficients r (Eq. 5) for important combinations of proxy scalars (CO_2 and T_s , CO_2 and H_2O). The abrupt decrease of the correlation after the management (see Fig. 7) suggests that both, T_s and H_2O are no suitable alternatives to CO_2 shortly after management. Moreover, a faster recovery of scalar similarity after autumn rowen (dark symbols) is indicated than after mid-summer mowing (bright symbols). This circumstance can be linked with greater intervention in the ecosystem in mid-summer, that is, removing more productive biomass than in autumn. In both cases, scalar similarity increased with ecosystem recovery up to pre-cutting values.

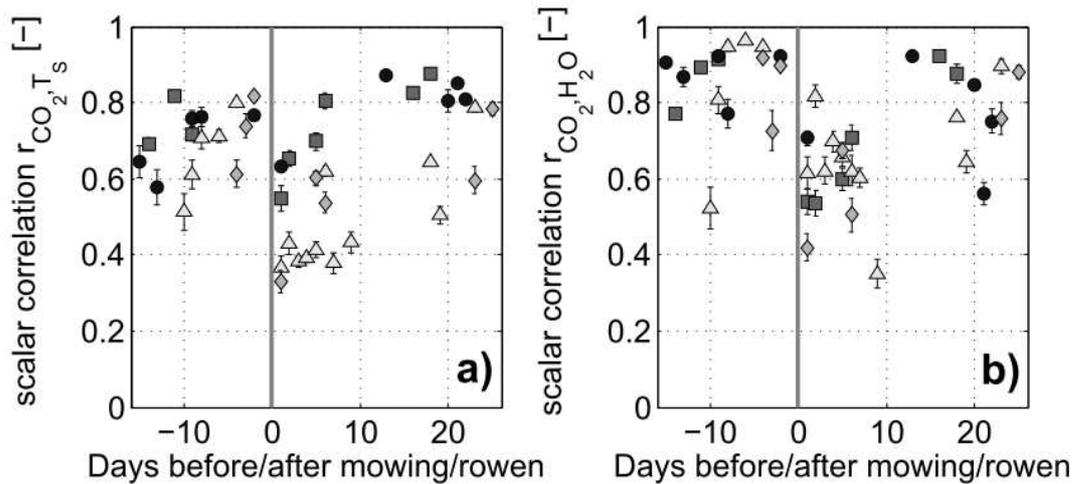


Figure 7. Scalar correlation coefficients between CO₂ and sonic temperature T_s (a) and CO₂ and water vapor H₂O (b). Negative x-axis values indicate days before, positive values days after mowing and rowen, respectively. Dark squares represent rowen data in September 2009, bright upward triangles mowing in July 2010, black circles rowen in September 2010 and grey diamonds the late mowing in August 2011. The error bars show standard errors of the daily mean of r between 09:00 and 17:00 CET.

A further impact on simulated b -factors was found, too. b showed a higher uncertainty and decreased strongly even to negative values after management. The detailed results are illustrated in Appendix B. As a consequence of this result, REA field experiments had been conducted with adequate time lag to the management events.

On 22 June and 25 August 2010, before and long after meadow management, all parameters were determined in order to partition the NEE into assimilation (F_A) and respiration (F_R), based on two different approaches: the commonly applied flux partitioning model (FPM), based on Lloyd Taylor and Michaelis Menten functions, and the isotopic flux partitioning approach (Eqs. 6 and 7). In general, both partitioning approaches correlated to a certain extent but some noticeable differences could be found (Fig. 8). Most obvious in this context is the difference in the last value in Figure 8a in the evening. While the morning rise of photosynthetic activity was missed, the evening breakdown to a respiration-dominated system could be sampled by REA. At that time, there is no longer any difference between up- and downdraft isotope ratios, so that isoflux, and consequently assimilation and respiration fluxes, become zero. This comes along with the lack of photosynthesis and discrimination, but, above all, with turbulent fluxes that come to a standstill, as it is confirmed by a very small NEE. This pattern is only shown by the isotope approach and not

by the Lloyd Taylor and Michaelis Menten function based FPM. Apart from that, the isotopic flux partitioning shows a much greater variability whereas the FPM reproduces natural respiration changes insufficiently, causing assimilation fluxes to follow the NEE exactly. Sometimes both approaches provide similar fluxes but the isotopic model is able to describe various underlying fluxes of the NEE; that is, more intense reactions to environmental conditions are attributed to the ecosystem (cf. Ruppert, 2008). The rather constant respiration which is provided by the temperature-based FPM, results from relatively small temperature variations during both periods around the measurement days. Temperature is only one of the driving mechanisms of respiration; for example, photosynthetic activity supplies root exudates to soil life and accounts for a large portion of root-derived respiration (Kuzyakov and Gavrichkova, 2010). Discrimination of ^{13}C is an input factor in the isotopic model. It is coupled directly to all assimilation-based processes. These become apparent with the assimilation flux which is closely connected to the incoming shortwave radiation. The same applies to wind velocity, essential for atmospheric fluxes and considered only in the isotopic model as an input parameter of the isoflux. In Appendix B, the dependence on those parameters is explained in detail.

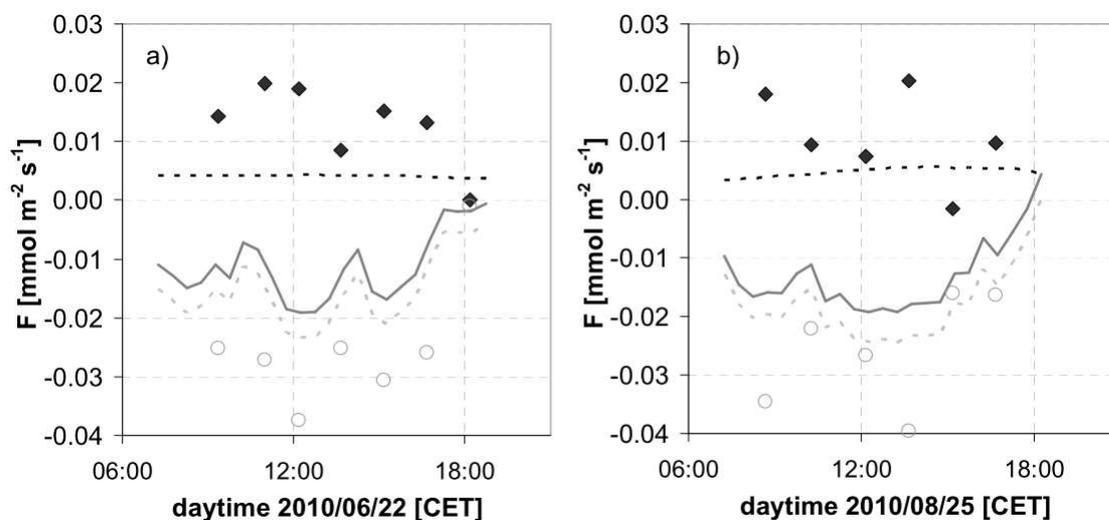


Figure 8. Comparison of NEE flux partitioning with isotopic background (respiration flux F_R : black diamonds, assimilation flux F_A : light grey circles) and a common FPM (dashed lines in same colors); the NEE measured by eddy-covariance is illustrated as dark grey solid line.

Isotopic flux determination by REA served another purpose related to the isotope labeling experiment. Only a very small portion of less than 1% $^{13}\text{CO}_2$ flux as part of the entire CO_2 flux was found on the grassland site. Thus, influences on isotopic tracer experiments can be excluded.

3.5 Partitioned absolute carbon fluxes into distinct carbon pools

As already mentioned, the ^{13}C recovery rate stabilizes at the end of the experiment (Fig. 4). There were no more significant changes in at least the last two samplings of each of the five pools in both variants. Hence, the precondition for the partitioning of the absolute carbon input, the isotopic steady state in the plant–soil–atmosphere system, was fulfilled (cf. Sagar and Hedley, 2001; Sagar et al., 1997; Wu et al., 2010) and the proportion of the tracer which was present at that time in the different pools could be offset against the total carbon input by assimilation, provided by eddy-covariance in combination with the flux partitioning model. This had been conducted exclusively for the 21 day tracing period after the pulse labeling since the transferability beyond this period was not validated by accounting for, for example plant physiological factors. The proportions of the inputs into the different pools follow naturally those in Figure 4. On average, $2.5\pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$ of the total input of $7.1\pm 0.4 \text{ g C m}^{-2} \text{ d}^{-1}$ were incorporated in the shoot and $0.8\pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ in the root biomass. $0.5\pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ remained in the soil whereas $2.3\pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ and $1.0\pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ were released to the atmosphere as shoot respiration and soil CO_2 efflux, respectively. As a sum, the two latter fluxes represent an ecosystem respiration (R_{ECO}) of $3.3\pm 0.4 \text{ g C m}^{-2} \text{ d}^{-1}$ which is predestined to serve as verification parameter since it was also determined independently of the labeling by separating the NEE by the flux partitioning model: $3.5\pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$ (Fig. 9). The good correlation indicates that this approach – coupling two methods – was applied successfully. Furthermore, comparable results based on other approaches to gain absolute carbon inputs could be found in the literature (Kuzyakov and Domanski, 2000; Appendix A).

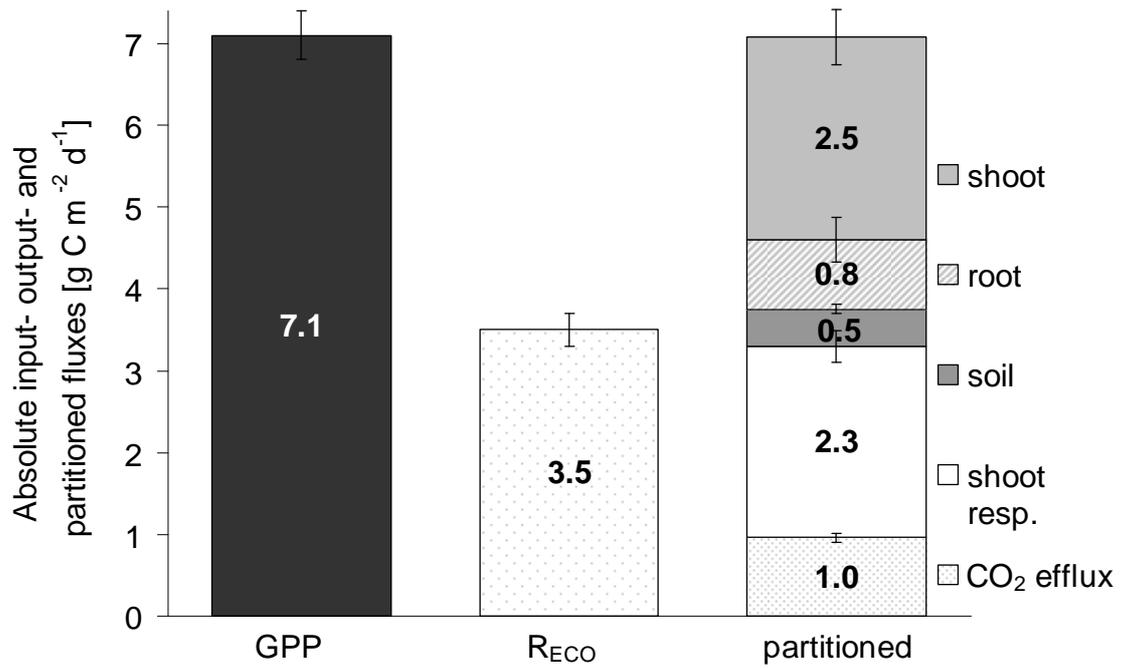


Figure 9. Average daily absolute input (GPP), output (R_{ECO}) and partitioned absolute carbon distribution after assimilation (g C m⁻² d⁻¹, \pm SEM) during the chase period of the labeling experiment. Please note that for illustration all values, even GPP, have a positive sign.

CONCLUSIONS

The main objective of this thesis is to advance investigation of the carbon cycle of extensively managed grassland – under consideration of contemporary changing environmental conditions (Bavarian State Ministry for Environment and Consumer Protection, 2013) – beyond commonly applied approaches. Achievements could be made regarding atmospheric measurement techniques as eddy-covariance and relaxed eddy accumulation (Appendix B), chamber measurements (Appendix C), stable isotope labeling (Appendix A) and flux partitioning approaches (all Appendices) by suggesting novel evaluation methods and combinations of those. From these achievements, the following conclusions can be drawn:

(i) Carbon sink and drought effects

Under the present environmental and site conditions, extensively managed grassland in mid European low mountain ranges recommends conservation and extension due to their mitigation capability in relation to carbon sequestration. If the grassland is mowed two times a year and even if the removed biomass is considered in the balance, there would still exist a sink capacity. The number of management events is relevant because a third cutting would not be compensated and the sink capability would disappear. In Bavaria, there are large areas of extensively managed grassland. Alteration of the land use is to avoid because carbon would be released to the atmosphere. In the investigated region, the changing climate can be observed in terms of spring drought events. Those alter the carbon allocation to different ecosystem compartments as roots and soil in the first instance. It is expected that the release of that carbon to the atmosphere is just delayed to the next precipitation event. In any case, further long term observation can be highly recommended as well as monitoring the influence of ground water at the region of interest. These factors will define future amounts and the quality of carbon sequestration.

(ii) Flux partitioning

Eddy-covariance is the preferential technique when to determine NEE. A deeper insight into the carbon cycle demands partitioning into gross primary production and ecosystem respiration. The representation of environmental influences as incoming shortwave radiation and wind velocity in combination with the accordance to the established common flux partitioning model based on Lloyd-Taylor and Michaelis-Menten functions, suggests a good performance of the isotopic partitioning model. It also works well on the grassland site

compared to former studies where it was applied over forest ecosystems with special coupling regimes (Ruppert, 2008; Wichura, 2009). For short term experiments, it can enhance results of the common flux partitioning tool which depend on a relatively long time series of temperature and radiation. This fact limits the sensitivity for reactions of the ecosystem on short term changes in the weather conditions. In contrast to that, the isotope based approach includes ecosystem discrimination of ^{13}C directly and, thereby, reproduces present environmental conditions in a better way. For partitioning and gap filling of long NEE datasets, the common flux partitioning model has to be preferred. REA application for isoflux determination in general is expensive and time consuming and is therefore applicable for short term and special investigations only. However, its versatility and the information about NEE component flux variability still justify its application in ecosystem sciences.

(iii) Chamber measurements

The evaluation of flux partitioning can also be accomplished by combined measurements of dark and transparent soil chambers. This works well during the day when atmospheric turbulence is established sufficiently. Then, state of the art chamber NEE measurements are in accordance with the results of eddy-covariance. During the diurnal cycle there are periods which suggest a contrary result. Chambers overestimate NEE during times of oasis effect in the late afternoon which is due to the fact that the microclimate in the chamber does not represent real atmospheric conditions but rather held the radiation and stratification conditions of the early afternoon up. During night, chambers miss present fluxes based on complex atmospheric phenomena as coherent structures. Due to their applicability on small scale experiments, chambers represent a valuable amendment in ecosystem sciences. However, the results have to be evaluated critically under consideration of the atmospheric framework conditions because those are often reproduced insufficiently (Dore et al., 2003; Lai et al., 2012; Rochette and Hutchinson, 2005).

(iv) REA application

As the influence of management events is important for the carbon sequestration potential, the same is true for some kinds of micrometeorological measurement techniques. Especially for the application of relaxed eddy accumulation which must not be applied shortly after the management due to serious consequences for the resulting fluxes. An essential determination of the reliable proportionality factor b is wrong due to lacking scalar similarity between the scalars shortly after the management. It is suggested to wait at least 22 days in summer and 12 days in autumn after the management in like circumstances.

Then, the ecosystem has normally recovered and the regular source/sink distribution (cf. Andreas et al., 1998a; Ruppert et al., 2006b; Held et al., 2008) and source strength (Katul et al., 1999; Katul and Hsieh, 1999) regarding the scalar quantities has normalized, too. In the literature these restrictions were implied (Williams et al., 2007) and observed in a comparable way for other micrometeorological methods (Businger, 1986).

(v) Partitioned absolute carbon input

First time coupling of atmospheric carbon flux measurements with ^{13}C pulse labeling worked well as the self-validation via ecosystem respiration and the comparison with few other methods presented in the literature suggest. The method combination allows a more detailed insight into the carbon cycle by providing absolute values of carbon input to distinct ecosystem compartments. One limitation is the restriction of the result to the sampling period shortly after the labeling. Due to the allocation pattern strongly depends on the stage of plant growth, weather conditions and management activities the result obtained by of a single pulse labeling cannot be transferred to a longer period (Gregory and Atwell, 1991; Kuzyakov and Domanski, 2000). However, a series of labeling pulses at regular intervals could provide reasonable estimates of the partitioning for the whole growth period (e.g. Kuzyakov and Schneckenberger, 2004). It is imaginable to conduct such an experiment in association with one of the numerous eddy-covariance measurement networks.

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LIST OF APPENDICES

The publications of this cumulative thesis are listed content-related and not chronologically.

A. RIEDERER ET AL. (2015)

B. RIEDERER ET AL. (2014b)

C. RIEDERER ET AL. (2014a)

D. INDIVIDUAL CONTRIBUTIONS TO THE JOINT PUBLICATIONS

A. RIEDERER ET AL. (2015)

Partitioning NEE for absolute C input into various ecosystem pools by combining results from eddy-covariance, atmospheric flux partitioning and $^{13}\text{CO}_2$ pulse labeling

M. Riederer ^{a,d,*}, J. Pausch ^b, Y. Kuzyakov ^b, T. Foken ^{a,c,†}

^a *Department of Micrometeorology, University of Bayreuth, D-95440 Bayreuth, Germany*

^b *Department of Soil Science of Temperate Ecosystems, University of Göttingen, D-37077 Göttingen, Germany*

^c *Member of Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D-95440 Bayreuth, Germany*

^d *now: Regensburg Center of Energy and Resources, Regensburg University of Applied Sciences, D-93049 Regensburg, Germany}*

* Corresponding author. Tel.: +49 941 943 1140, Fax: +49 941 943 1424

e-mail: michael.riederer@oth-regensburg.de

† Corresponding author. Tel.: +49 921 55 2293, Fax: +49 921 55 2366

e-mail: thomas.foken@uni-bayreuth.de

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 eddy-covariance and stable isotope labeling – obtained in a 21-day experiment in summer 2010 – were combined. Eddy-covariance based net ecosystem exchange was measured on extensively managed grassland and separated into underlying assimilation and respiration through the use of a C flux partitioning model. Resultant CO₂ assimilation served as absolute C input into the ecosystem and was further partitioned by applying the relative C distribution in subsidiary pools, gained by ¹³C pulse labeling and tracing.

Results

The results from eddy-covariance measurements 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 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 CO₂ efflux from soil, respectively.

Conclusions

We conclude that the combination of EC measurements and atmospheric flux partitioning 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.

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; Aubinet et al. 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 e.g. 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, the 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; Buchmann 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, 2003; 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 input that is representative for the

whole ecosystem. Combining the results of EC with that of $^{13}\text{CO}_2$ 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), while 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 CO_2 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 $^{13}\text{CO}_2$ pulse labeling experiment were conducted. To our knowledge, this is the first study combining results of EC measurements and of a CO_2 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. ($50^{\circ}05'25''\text{N}$, $11^{\circ}51'25''\text{E}$) 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), 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 minute 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; Mauder et al. 2007; Eigenmann et al. 2009). The included quality flagging system evaluated stationarity and turbulence during the averaging interval of 30 minutes 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; Göckede et al. 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 minute 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).

¹³C pulse labeling for determination of relative proportion of C partitioning

Experiment setup

Five stainless steel soil frames (each 1 x 1 m²) with a u-shaped bar at the upper end were inserted up to 10 cm depth three weeks prior to labeling in order to reduce disturbances. For ¹³CO₂ pulse labeling the upper part of the chamber, consisting of aluminum frames (base of the frame 1 x 1 m², height 0.5 m) were placed into the u-shaped bar which was filled with water (containing a small amount of H₂SO₄) 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: ~90%) shortly before the tracer addition. To minimize the influence of the chamber on the tracer uptake, five cooling aggregates (EZetil Iceakku, 220g), 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 ¹³C tracer as Na₂¹³CO₃ (5 g 99% ¹³C-enriched Na₂CO₃), was placed behind the fan to assure homogenous distribution of the labeled CO₂. An excess of 5 M H₂SO₄ was added to the tracer solution from outside the chamber with a syringe. The puncture holes 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 three hours to assure complete uptake of the ¹³CO₂. To avoid noon depression of photosynthesis, labeling was conducted from 2:30 pm – 5:30 pm. In one of the chambers the CO₂ 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 ¹³CO₂ (McDermitt et al. 1993), but the concentration increased at the beginning up to 1500 ppm and a concentration next to zero after the 3 hours was measured indicating the complete uptake of the tracer. Shortly before the labeling the CO₂ concentration within the chamber dropped down to zero due to

assimilation. It is expected that this very short lack of CO₂ and the high concentration after adding the tracer had no noticeable influence on the experiment. The reasons for that are explained in the following chapters.

Data acquisition and analysis

Translocation of the assimilated ¹³C was analyzed during a 21-day period in shoots, roots, soil and soil CO₂ 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 0–30 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 δ¹³C natural abundance for calculations. All samples were frozen (-20°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 δ¹³C (‰) 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 CO₂ 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 (inner diameter 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 was 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 $\delta^{13}\text{C}$ (‰) values, SrCO_3 was precipitated with SrCl_2 , neutralized and dried for the EA-IRMS measurements. For the calculation of the relative proportion of ^{13}C input into various pools (shoots, roots, soil and CO_2 efflux were investigated) after $^{13}\text{CO}_2$ pulse labeling several calculation steps were necessary. The enrichment of ^{13}C in a C pool ($x^E(^{13}\text{C})$, atom%) was derived by subtracting the naturally abundant amount of ^{13}C ($x(^{13}\text{C})_{std}$, atom%) from the amount of ^{13}C in the labeled pool P ($x(^{13}\text{C})_p$, atom%):

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

where E marks the excess on ^{13}C 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 $\delta^{13}\text{C}$ value of soil CO_2 efflux, measured beside the labeling plots, was determined by correcting the measured $\delta^{13}\text{C}$ values for the admixture of atmospheric CO_2 , based on the Miller/Tans model (Miller and Tans 2003; Pausch and Kuzyakov 2012). Therefore, measured $\delta^{13}\text{C}$ values multiplied by the respective CO_2 concentrations were plotted against the CO_2 concentrations. The slope of the regression line is equivalent to the $\delta^{13}\text{C}$ value of soil CO_2 efflux purified from atmospheric CO_2 (Miller and Tans 2003). The Miller/Tans model was applied in combination with a geometric mean regression (GMR), as suggested for soil CO_2 (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 ^{13}C amount ($n(^{13}\text{C})_p$, g ^{13}C m⁻²) of the pool was calculated:

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

Since all calculations were carried out with area units 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 ^{13}C during the sampling period, the total amounts of ^{13}C found immediately after the labeling ($n(^{13}\text{C})_{P_{t_0}}$, $\text{g } ^{13}\text{C m}^{-2}$) were summed up over all investigated pools. Then the ^{13}C amounts of every single pool at every point of time ($n(^{13}\text{C})_{P_t}$, $\text{g } ^{13}\text{C m}^{-2}$) could be related to this total value and the recovery (R , %) of the tracer could be calculated using the equation:

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

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 ^{13}C amount ($n(^{13}\text{C})_P$, $\text{g } ^{13}\text{C m}^{-2}$) within the CO_2 efflux ($F_{\text{CO}_2, \text{soil}}$) 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 ^{13}C was considered in that pool as well. The losses of ^{13}C by shoot respiration were not measured, but could be estimated by the following equation:

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

Due to translocation to deeper soil layers was investigated and excluded, it is assumed that shoot respiration is the only relevant missing sink of ^{13}C within the considered system, the ^{13}C 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 during the sampling period. 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

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

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 grow 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. Fig. 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).

¹³C dynamics and allocation

The sampling immediately started after the ¹³CO₂ tracer was completely assimilated. The ¹³C 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 ¹³C was translocated from shoots into roots directly after labeling. The ¹³C recovery of roots did not change significantly over 21 days. In contrast to the roots, the maximum ¹³C amount of the soil pool was detected one day after the labeling. Thereafter, the ¹³C recovery in the soil slightly decreased and reached 6.4% 21 days after labeling (Fig. 2b). Similar to shoot respiration, ¹³C in soil CO₂ efflux was highest during the first day and then declined over time.

The allocation of ¹³C tracer was mostly completed after 9 days and the ¹³C 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. Fig. 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 CO₂ efflux, dominates the proportion by accounting for almost half (46.7%) of the assimilated ¹³C. About one third (34.9%) remains in the shoots, while roots and soil obtain, with 12% and 6.4%, respectively, comparatively small proportions of ¹³C. Overall about 32% of assimilated ¹³C 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 partitioned for absolute C allocation into individual pools based on the ¹³C recovery of the respective pool. The ¹³C 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 $2.3 \pm 0.3 \text{ g C m}^{-2} \text{ d}^{-1}$ and $1.0 \pm 0.1 \text{ g C m}^{-2} \text{ d}^{-1}$ were released to the atmosphere as shoot respiration and soil CO₂ efflux, respectively. The sum of the soil CO₂ 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 $3.5 \pm 0.2 \text{ g C m}^{-2} \text{ d}^{-1}$, determined independently by separating the NEE with the FPM (Fig. 3).

Discussion

Discussion overview

By combining the results of atmospheric CO₂ flux measurements and ¹³CO₂ pulse labeling, a new approach for partitioning ecosystem C fluxes was introduced. In the following, the results will be discussed in detail. Absolute atmospheric CO₂ fluxes will be compared to further flux measurements under similar environmental conditions, and relative assimilate distribution will be compared to those of other ¹³C 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 CO₂ fluxes on such grasslands at elevations from 375 to 1770 m a.s.l., with mean annual temperatures from 5.5 to 9.5 °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 $481 \text{ g C m}^{-2} \text{ a}^{-1}$ 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 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 ^{13}C allocation

Isotopic pulse labeling was used to quantify the input of ^{13}C 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 ^{13}C 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 two 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 can be also an explanation for the non-significant changes 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 two 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 or 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 one 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).

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 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). In contrast, a series of labeling pulses at regular intervals (Keith et al. 1986; Swinnen et al. 1994; Kuzyakov et al. 1999; Kuzyakov et al. 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 eddy-covariance 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 combination: further separation of the NEE beyond assimilation and respiration fluxes is provided and labeling approaches are upgraded by finally dealing with absolute instead of relative C allocation. 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.

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Tables

Table 1. Annual ($\text{g C m}^{-2} \text{ a}^{-1}$) and daily ($\text{g C m}^{-2} \text{ d}^{-1}$) C fluxes ($\pm\text{SEM}$) 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 | | |

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 g C m⁻² 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 | Grillenburg, 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 | Alinyà, 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 | <u>This study</u> |
| 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) |

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 _{ECO} | 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*, % 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**, % of assimilated*** | | 27.0 | | 5.0 | 14.0 | 54.0 | | | (Davenport and Thomas 1988) |
| 11 | 21 agric.plants Review / % of net assimilated**** | | 60 | 36 | 19 | 5 | | | 12 | (Jones et al. 2009) |
| 12 | Pasture plants, Review / % of assimilated*** | | 30 | 40 | 20 | 5 | 45 | 30 | 15 | (Kuzuyakov 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 |

- * Continuously labeled with ^{14}C during the whole time of growing
- ** Weekly repeated pulse labeling, as suggested to cover whole growth period
- *** Recovery related to assimilated amount of tracer (shoot respiration is not considered, may underestimate final result by up to 30%)
- **** Recovery related to net assimilated amount of tracer (shoot respiration is considered)

Figures

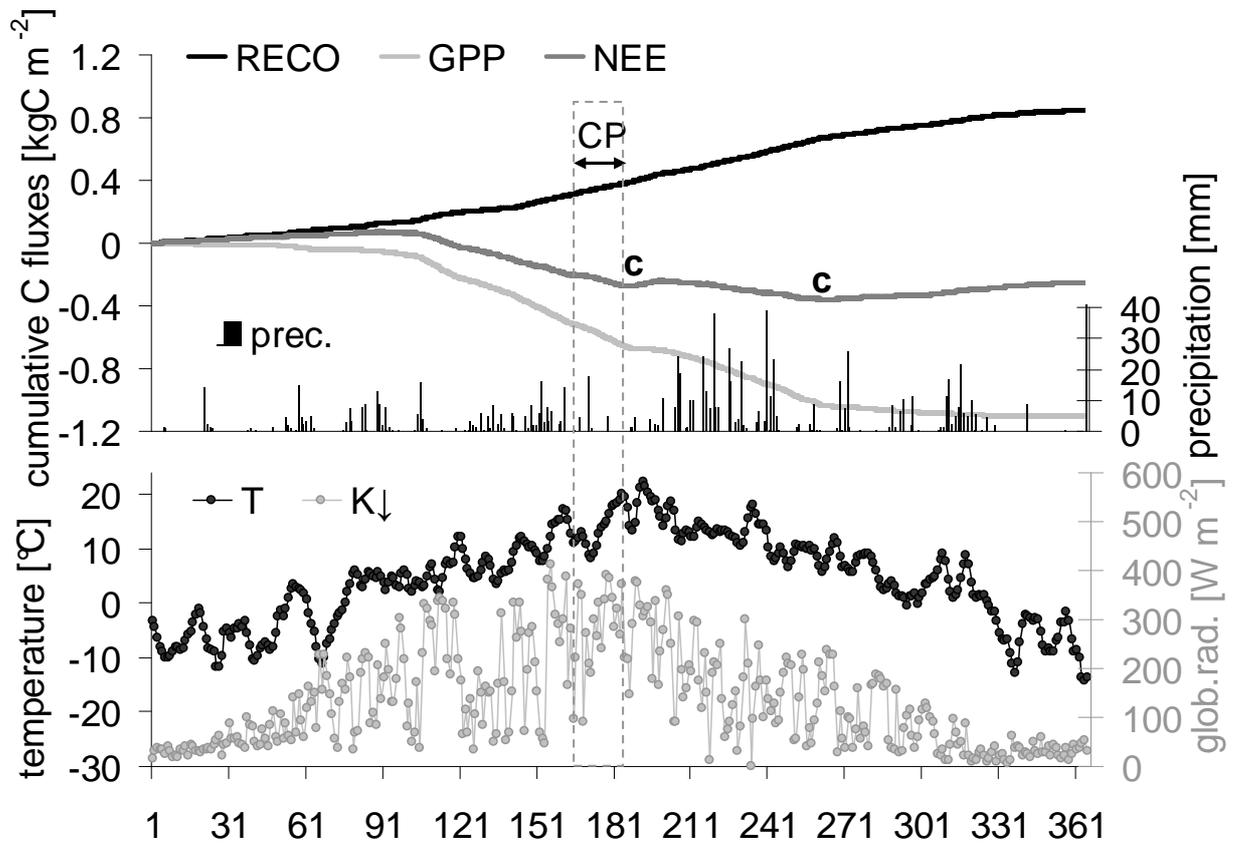


Fig. 1. Cumulative annual fluxes of NEE, GPP and R_{ECO} (flat lines), daily sums of precipitation (black bars), daily means of global radiation (grey filled circles) and daily mean temperatures (black filled circles). The box with dashed outline begins with the pulse labeling and comprises the chase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

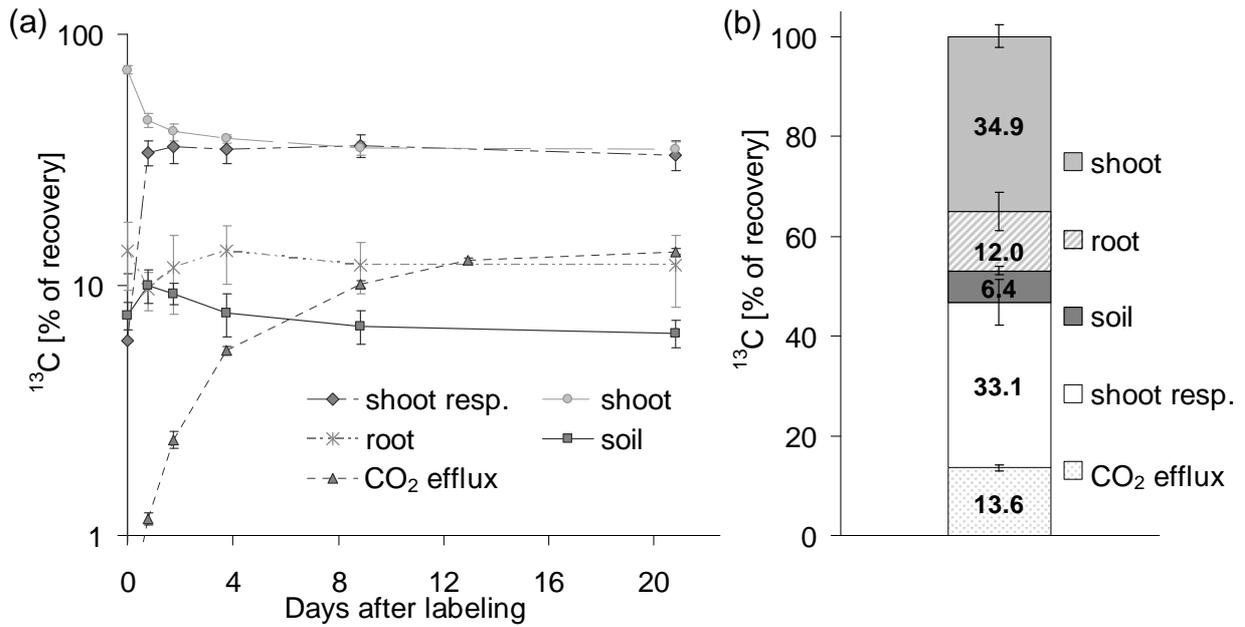


Fig. 2. ^{13}C dynamics during the chase period in all C pools (a) and relative proportion of ^{13}C recovered, i.e. final distribution by percentage at the last day of sampling (day 21 of the chase period) in the ecosystem C pools (b). The x-axis of (a) intersects at $y = 1\%$ for a better illustration. 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 ($\pm\text{SEM}$).

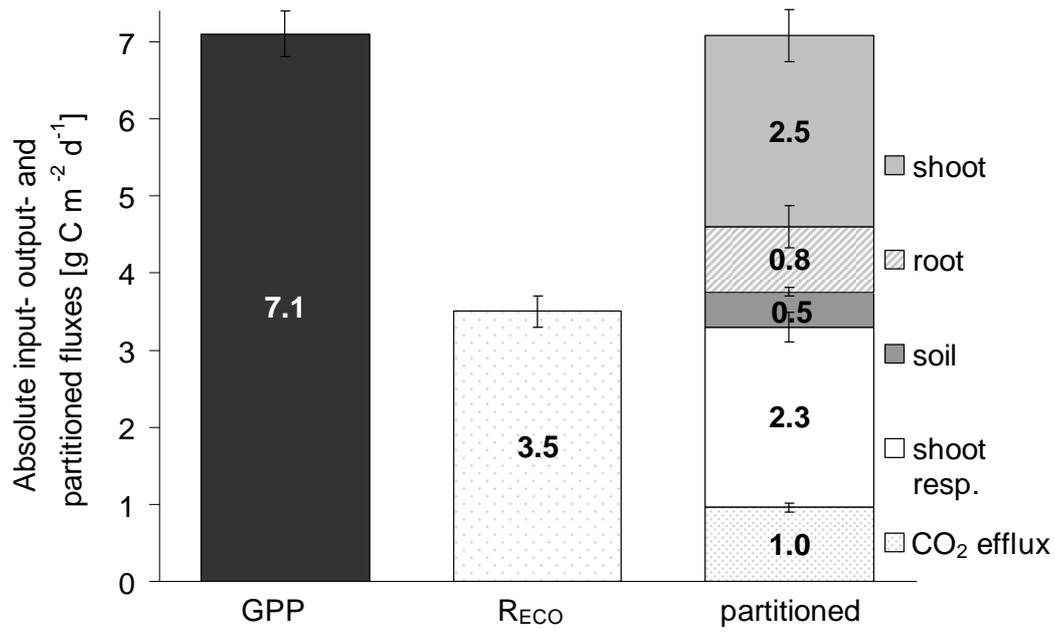


Fig. 3. Average daily absolute input (GPP), output (R_{ECO}) and partitioned absolute C distribution after assimilation ($\text{g C m}^{-2} \text{d}^{-1}$, $\pm\text{SEM}$) during the chase period of the labeling experiment. Please note that for illustration all values, even GPP, have a positive sign.

B. RIEDERER ET AL. (2014b)

Prerequisites for application of hyperbolic relaxed eddy accumulation on managed grasslands and alternative NEE flux partitioning

M. Riederer^{1,*}, J. Hübner¹, J. Ruppert^{1,}, W.A. Brand², T. Foken^{1,3}**

[1] {Department of Micrometeorology, University of Bayreuth, D–95440 Bayreuth, Germany}

[2] {Max Planck Institute for Biogeochemistry, D–07745 Jena, Germany}

[3] {Member of Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D–95440 Bayreuth, Germany}

[*] {now: Regensburg Center of Energy and Resources, Regensburg University of Applied Sciences, D–93049 Regensburg, Germany}

[**] {now: Research Institute of the Cement Industry, D–40410 Düsseldorf, Germany}

Correspondence to: M. Riederer (michael.riederer@oth-regensburg.de)

T. Foken (thomas.foken@uni-bayreuth.de)

Abstract

Relaxed eddy accumulation is still applied in ecosystem sciences for measuring trace gas fluxes. On managed grasslands, the length of time between management events and the application of relaxed eddy accumulation has an essential influence on the determination of the proportionality factor b and thereby on the resulting flux. In this study this effect is discussed for the first time. Also, scalar similarity between proxy scalars and scalars of interest is affected until the ecosystem has completely recovered. Against this background,

CO₂ fluxes were continuously measured and ¹³CO₂ isofluxes were determined with a high measurement precision on two representative days in summer 2010.

Moreover, a common method for the partitioning of the net ecosystem exchange into assimilation and respiration based on temperature and light response was compared with an isotopic approach directly based on the isotope discrimination of the biosphere. This approach worked well on the grassland site and could enhance flux partitioning results by better reproducing the environmental conditions.

1 Introduction

In ecosystem sciences the interface of atmosphere, biosphere and soil is of particular importance and affords insight into the carbon (C) cycle from various angles of view with various methods that, in turn, have to be adapted and evaluated. Insight from an atmospheric perspective is mostly achieved by chamber (Goulden et al., 1996; Davidson et al., 2002; Dore et al., 2003; Subke and Tenhunen, 2004) and micrometeorological techniques as e.g. eddy-covariance (EC; Moncrieff et al., 1997; Aubinet et al., 2000; Aubinet et al., 2012; Baldocchi et al., 2001; Baldocchi, 2003). While chambers are able to directly measure ecosystem carbon source and sink fluxes on small spatial scales, EC provides net ecosystem carbon exchange (NEE) on larger spatial scales. More detailed information about underlying fluxes can be achieved by applying flux partitioning models (FPMs, Stoy et al., 2006; Desai et al., 2008; Lasslop et al., 2010; Reichstein et al., 2012). Those are also used to gap-fill missing or excluded flux data (Falge et al., 2001; Stoy et al., 2006; Ruppert et al., 2006a; Desai et al., 2008; Papale, 2012). Those models may provide fluxes with unrealistic temporal variation or magnitude (Stoy et al., 2006). However, an alternative is partitioning based on additional determination of the ¹³CO₂ isoflux (Yakir and Wang, 1996; Bowling et al., 2001; Knohl and Buchmann, 2005; Ogée et al., 2004; Wichura, 2009; Wichura et al., 2004; Ruppert, 2008; Lloyd et al., 1996). Due to stomatal uptake of CO₂ and photosynthesis of C3 vegetation discriminate against the heavier isotope ¹³C, the biomass and all following compartments within the dynamic C-cycle are ¹³C depleted with respect to the atmosphere. Therefore the ¹³C isotopic ratio increases in the air during the assimilation period. Both effects account for a distinct diurnal cycle of the ¹³CO₂ mixing ratio in ecosystem air (Flanagan et al., 1996; Lloyd et al., 1996). Resulting

$^{13}\text{CO}_2$ isofluxes can be determined with the flux-gradient method (Flanagan et al., 1996), by modeling approaches (Ogée et al., 2003; Lloyd et al., 1996), by hyperbolic relaxed eddy accumulation (HREA, Bowling et al., 2001; Bowling et al., 2003a; Wichura, 2009; Wichura et al., 2004), the hybrid eddy covariance / flask gradient method (Bowling et al., 1999a; Griffis et al., 2004) and direct isotopic eddy covariance (Griffis et al., 2008; Sturm et al., 2012; Wehr et al., 2013).

This study examines the application of the REA-method on managed grasslands. European grassland ecosystems are still to be defined as a net carbon sink or source. For this reason, detailed investigation is strongly required (Ciais et al., 2010; Soussana and Lüscher, 2007). In addition to the general uncertainty regarding the sink/source behavior of grasslands, management of grassland ecosystems causes anomalies in the seasonal carbon cycle (Flechard et al., 2005). Such events have to be observed carefully, but potentially introduce certain restrictions for REA measurements. Previous studies indicated that errors in the REA flux often appeared when scalars of interest and proxy scalars behaved differently in their turbulent transportation efficiency (Ruppert et al., 2006b). This so-called scalar similarity is especially required for hyperbolic REA, because two important factors – the hyperbolic deadband H and the proportionality factor b , and consequently the REA flux – will be flawed without it (Oncley et al., 1993; Ruppert et al., 2006b). Even if b -factors are often treated as constant (Meyers et al., 2006; Haapanala et al., 2006), they show a certain diurnal variation. Other studies on managed ecosystems apply CO_2 and water vapor (Baum and Ham, 2009) and mostly temperature (Myles et al., 2007; Hensen A. et al., 2009) as proxy scalars, sometimes shortly after the management (Nemitz et al., 2001). Whether this practice can be problematic was thoroughly investigated in this study by numerous simulations with data from mown and unmown grassland. The real REA measurements of this study were conducted – in correspondence with the results of the simulation – before (22 June), and a sufficient period of time after the mowing (25 August) in the main growth period of 2010. The reasons for this procedure will be explained in the following, especially in section 4.1. Another aim of this study is to evaluate a commonly applied partitioning method for the net ecosystem exchange with a small number of the labor-intensive and sophisticated REA measurements. Ruppert (2008) and Wichura (2009) investigated an isotopic approach by Lloyd et al. (1996) for partitioning the net ecosystem exchange (NEE) above forest ecosystems, and found some restrictions due to complicated

coupling conditions (Thomas and Foken, 2007). In the current study, this approach is tested above grassland and finally evaluated by comparison with a common flux partitioning model (FPM) based on the temperature dependence of respiration after the Lloyd-Taylor function (Lloyd and Taylor, 1994). For R_{ECO} parameterization, nighttime NEE is used because it equals ecosystem respiration (R_{ECO}) due to missing assimilation (Lloyd and Taylor, 1994; Falge et al., 2001; Ruppert et al., 2006a; Ammann et al., 2007; Reichstein et al., 2005). To parameterize daytime solar radiation dependant gross primary production (GPP, Falge et al., 2001; Ruppert et al., 2006a), light response regression based on Michaelis-Menten function (Michaelis and Menten, 1913) was applied. Instead of a temperature binning approach a time window scheme was applied, which is preferred for sites with distinct seasonal variation or treatments generating abrupt changes in ecosystem behavior (Ammann et al., 2007).

In summary, this paper comprises the examination of REA application on managed grasslands by comparing scalar similarity and the b factor before and after management events. Then two real REA measurements were conducted in correspondence with the results of the simulation. The results of those were used to test an isotopic flux partitioning approach above grassland and to evaluate it by comparison with common flux partitioning.

2 REA theory and framework

The basic idea of Desjardins in 1972 (Desjardins, 1977) of separating the vertical wind into an up- and downward component was applied by Businger and Oncley (1990). They combined this eddy accumulation method (EA) with the flux-variance-similarity and created an indirect method: the relaxed eddy accumulation (REA), with the REA-flux

$$F_{REA} = b \sigma_w \rho_a (\overline{c_\uparrow} - \overline{c_\downarrow}) \quad (1)$$

derived from average up- and downward mixing ratio of the scalar $\overline{c_\uparrow}$ and $\overline{c_\downarrow}$, standard deviation of the mean vertical wind velocity σ_w , density of dry air ρ_a and an empirical and dimensionless proportionality factor b that compensates for the loss of information due to the mentioned “relaxation” (Ruppert et al., 2006b). Air samples are thereby taken with a constant flow rate and are not weighted according to the vertical wind speed (Businger and Oncley, 1990). b is often suggested to be unaffected by the atmospheric stability (Businger

and Oncley, 1990; Delany et al., 1991; Foken et al., 1995) but it can also depend on it (Andreas et al. 1998b). For an ideal Gaussian joint frequency distribution (JFD) of the vertical wind speed and the mixing ratio of the scalar, b is constant ($b = 0.627$; Baker et al., 1992; Wyngaard and Moeng, 1992). But variations of b can be generated by just a small skewness of the JFD of w' and c' (Katul et al., 1996; Fotiadi et al., 2005; Ruppert et al., 2006b), the application to different trace gases (Businger and Oncley, 1990; Baker et al., 1992; Oncley et al., 1993; Pattey et al., 1993) or the use in different ecosystem compartments (Gao, 1995). b may also vary significantly during individual 30-min integration intervals (Businger and Oncley, 1990; Baker et al., 1992; Oncley et al., 1993; Pattey et al., 1993; Beverland et al., 1996; Katul et al., 1996; Bowling et al., 1999a; Ammann and Meixner F.X, 2002; Ruppert et al., 2006b). Variations from 0.54 to 0.60 were found for several experimental data. Consequently, to reduce relative errors in flux determination, individually simulated b -values for every measurement location and period are to be favored over application of a constant b (Ruppert et al., 2006b; Foken, 2008). Thus, b is determined individually by REA simulation of a proxy scalar, which can be additionally measured by EC. The proportion of both proxy scalar fluxes, F_{REA} and the EC flux ($F_{EC} = \overline{w'c'}$), provides b :

$$b = \frac{\overline{w'c'}}{\rho_a \sigma_w (\overline{c_{\uparrow}} - \overline{c_{\downarrow}})} \quad (2)$$

Above-mentioned b -values were determined in theoretical simulations, where it is possible to separate up- and downdrafts exactly by sign, down to the smallest values. Not until Businger and Oncley (1990) modified the method by discarding fluctuations around zero – that have only small influences on the entire flux anyhow – with a deadband, could the REA idea be implemented with regard to mechanical restrictions of the speed of valve switching.

$$b(w_0) = \frac{\overline{w'c'}}{\rho_a \sigma_w (\overline{c_{\uparrow}}(w > w_0) - \overline{c_{\downarrow}}(w < -w_0))} \quad (3)$$

The size of the deadband w_0 around zero is determined individually according to experimental conditions and the particular scalar of interest. The same applies to $b(w_0)$, which has to be determined individually by conducting simulations with proxy scalars.

To maximize mixing ratio difference between up- and downdraft air samples, application of a hyperbolic deadband H is recommended (Bowling et al., 1999b). That is required for investigating, for example, differences in the order of the measurement precision. By application of H in hyperbolic REA (HREA), $b(H)$ is reduced to lower values around 0.22 ± 0.05 (Bowling et al., 1999b), 0.15-0.27 (Ruppert et al., 2006b), respectively. H is based on the fluctuation of the vertical wind velocity w' and the mixing ratio c' of a proxy scalar, as well as their standard deviations σ_w and σ_c :

$$\left| \frac{w'c'}{\sigma_w \sigma_c} \right| \geq H \quad (4)$$

Real time knowledge of σ_w and σ_c was achieved, by continuous online recalculation from the most recent 6 min of data. Those were weighted by applying a linear function by which the newest data was rated three times more important than the oldest data (Ruppert et al., 2012). Adequate scalar similarity is especially required for hyperbolic REA, because H depends on the mixing ratio of the proxy scalar. Generally, $b(H)$ and the REA flux as the final product will be flawed without scalar similarity between the scalar of interest and the proxy scalar (Oncley et al., 1993; Ruppert et al., 2006b). Scalar similarity was defined by Kaimal et al. (1972) and Pearson et al., (1998) as similarity in the scalar time series over all the scalar spectra. This means that scalar quantities are transported with similar efficiency by turbulence elements of diverse characteristics (Ruppert et al., 2006b). Differences in distribution (Andreas et al., 1998a; Ruppert et al., 2006b; Held et al., 2008), amount, and strength (Katul et al., 1999; Katul and Hsieh, 1999) of scalar sources and sinks are reasons for differences in turbulent exchange of the scalars and for lacking scalar similarity. Furthermore, scalar similarity is influenced by the seasonal variation of canopy physiology (Williams et al., 2007).

Essential for successful REA application is the choice of the right proxy scalar. Often temperature, measured by a sonic anemometer, is used (Graus et al., 2006; Lee et al., 2005; Grönholm et al., 2007; Bash and Miller, 2008; Bowling et al., 1998; Gaman et al.; Ren et al., 2011) and sometimes both temperature and water vapor turn out to be adequate (Held et al., 2008). For $^{13}\text{CO}_2$ isoflux measurements typically the proxy scalar CO_2 is used (Bowling et al., 2003a; Wichura, 2009). Due to a lack of adequate high frequency measurements of $^{13}\text{CO}_2$, scalar similarity between both cannot be evaluated, but it is

assumed that both scalars behave similarly. However, sources and sinks are quite different in strength and distribution with respect to discrimination against ^{13}C during assimilation (Bowling et al., 2003a).

3 Material and methods

3.1 Study area

All experiments relevant for this study were conducted on the extensively managed submontane grassland site “Voitsumra” at the edge of the low mountain range “Fichtelgebirge” in northeast Bavaria, Germany, located 624 m a.s.l. (50°05′25″N, 11°51′25″E). For the last 10 years the site has been used as extensively managed grassland without fertilization or grazing, but with sporadic mowing once or twice a year. The plant community is described as *Molinio-Arrhenatheretea* R. Tx. 1937 – economic grassland (Oberdorfer, 2001) and the most dominant of the 48 species are *Alchemilla monticola*, *Juncus filiformis*, *Polygonum bistorta*, *Ranunculus acris* and *Trifolium repens*.

3.2 EC measurements

High frequency data for both EC and REA were collected 2.5 m above ground at 20 Hz. Water vapor and CO_2 mixing ratio were measured by an open-path gas analyzer (LI-7500, LI-COR Biosciences, Lincoln, Nebraska USA), and wind vector and sonic temperature (T_s) by a 3D sonic anemometer (CSAT3, Campbell Scientific, Inc., Logan, Utah USA). All turbulence data were stored on a data logger (CR3000, Campbell Scientific, Inc., Logan, Utah USA) and collected daily by a micro-computer system (MICRO-ITX, CarTFT.com) as a backup. To avoid inconsistencies in the wind vector time series, the inclination of the CSAT3 was monitored by an inclinometer on the top of the measurement mast. The computation of the EC-flux was accomplished by the software package TK2 developed at the University of Bayreuth (Mauder and Foken, 2004); TK3 has become available in the meantime (Mauder and Foken, 2011). This software contains all necessary data correction and data quality assessment tools (Foken et al., 2012b) and was approved in comparison with six other commonly used software packages (Mauder et al., 2008) and successfully

applied in considerable field campaigns (Mauder et al., 2006; Mauder et al., 2007; Eigenmann et al., 2009).

Data processing for determining turbulent EC-fluxes begins with spike and outlier filtering (Rebmann et al., 2012). Data can then already be used for REA-simulation. All following steps in TK2 relating to determination, quality testing and validation of the EC-flux, are explained in detail by Mauder and Foken (2004), Foken (2008) and Foken et al. (2012a). This also includes planar fit correction (Wilczak et al., 2001), which was also applied in the REA controlling software (Ruppert, 2005). Wind velocity datasets of the four weeks before each REA field measurement were analyzed and planar fit corrections were implemented in the REA controlling software (Ruppert, 2005). Due to very small rotation angles only minor corrections were necessary. To assure that the signal measured by EC originated exclusively from the target land use type “grassland”, footprint analysis was performed (Göckede et al., 2004; Göckede et al., 2006; Rannik et al., 2012). It has been proven that more than 95% of the data originated from grassland and were not influenced by surrounding land use types such as tracks and creeks.

3.3 Simulation for $b(H)$ and H

Due to the great importance of the b -factor for proper REA flux determination, and especially due to the lack of information about possible effects of management events on grasslands, the variation of $b(H)$ was investigated by simulation. Therefore, a two-year eddy covariance data set (2010, 2011) and accompanying low frequency measurements of meteorological parameters were available. Hence, an adequate number of days with similar atmospheric conditions could be chosen within pre- and post-mowing periods to secure better comparability and to focus exclusively on the effects induced by management. Also the variation of $b(H)$ within the diurnal cycle was evaluated.

However, the first step was to determine the hyperbolic deadband H . The size of H was defined in advance and adapted according to the outcome of b . Finally, in combination with former studies as a reference (Ruppert, 2008), the hyperbolic deadband was defined constant as $H = 1$. During the simulation, the sign of the fluctuation of the vertical wind component w' determines partitioning of the scalar of interest in up- and downdraft, just as during real REA sampling. In this case, the CO_2 mixing ratio was used as proxy scalar.

The difference of the separately summed proxy scalar values $(\overline{c_{\uparrow}} - \overline{c_{\downarrow}})$ and the standard deviation of the vertical wind speed σ_w were calculated. By comparison of the product $\sigma_w(\overline{c_{\uparrow}} - \overline{c_{\downarrow}})$ to the corresponding EC-flux $\overline{w'c'}$ – determined by high frequency measurements and TK2-software – individual factors $b(H)$ could be derived (Eq. 3). That evaluation was also applied to other commonly used proxy scalars such as T_S and water vapor (H_2O). In order to evaluate their similarity to CO_2 in the scalar time series, i.e. their suitability as a substitute for CO_2 , scalar similarity had to be verified, and this was done by evaluating the consistency of the correlation coefficients r . Those coefficients were calculated for the combinations of the scalars $CO_2 - H_2O$ and $CO_2 - T_S$ by the following equation

$$r_{c_{proxy1}, c_{proxy2}} = \frac{\overline{c'_{proxy1} c'_{proxy2}}}{\sigma_{c_{proxy1}} \sigma_{c_{proxy2}}} \quad (5)$$

as already applied in other studies with the correlation coefficient ranging from zero (no correlation) to one (full correlation; Gao, 1995; Katul and Hsieh, 1999; Ruppert et al., 2006b; Held et al., 2008). To figure out possible differences before and after the management daily average correlation coefficients (between 9:00 and 17:00, for 16 days before and 27 days after the management) were calculated.

3.4 REA preparation and measurements

The REA device used in this study (Fig. 1) was constructed and tested by Ruppert et al. (2012) and has already been applied in other field experiments (Ruppert, 2008). REA measurements require high frequency vertical wind velocity and CO_2 mixing ratio, provided by an ultra-sonic anemometer and an infra-red gas analyzer. According to the hyperbolic deadband (Eq. 4), these data control the mechanical valve system for taking up- and downdraft air samples. The filter-protected intake line of the system, tested and optimized for time lag and turbulent flow (Reynolds number = 2433; Ruppert et al., 2012), was installed close to the measurement path of the sonic anemometer. The time lag between the air sample in the tube and the signal of the sonic anemometer was determined beforehand by cross correlation analysis and provided in the REA controlling software for

online correction (Ruppert, 2005). Valve switching for the separation of up- and downdrafts (located in the REA device) was thereby synchronized with the vertical wind velocity fluctuation, measured by the sonic anemometer (located 2.5 m above the ground).

All system components were either thoroughly cleaned with Acetone:Hexane 1:1 (nanograde) and heated (glass and steel parts), or possess non-reactive surface materials as Teflon[®] or Polyethylene to avoid fractionation processes and sample contamination. Mylar[®] balloons, also with Polyethylene as the inner surface, were used as intermediate sample reservoirs at ambient pressure. Isotopic integrity for up to a residence time of 60 minutes in the balloons could be achieved by repeated flushing and heating before application (cf. Bowling et al., 2003a and Ruppert et al., 2012). After a final leakage test the REA system was applied in the field. There was no need for density corrections, because the sample air was pre-dried with a Nafion[®] gas-dryer and finally dried by passing water traps with magnesium perchlorate granulate (Mg(ClO₄)₂). Between two sampling processes the system was flushed extensively with dry air from the measurement height to avoid any leftover sample air from previous sampling. More than 10 L up- and downdraft air were collected during each sampling process. Hence, the whole system, including sampling flasks for final storage for laboratory analysis, was repeatedly flushed and conditioned with dried air to achieve high sampling accuracy for subsequent high precision isotope ratio mass spectrometry (IRMS) analysis (Brand, 2005; Rothe et al., 2005; Sturm et al., 2004). This, as well as the CO₂ mixing ratio analysis, were accomplished in the isotope and trace gas laboratory of the Max-Planck Institute in Jena, Germany. All ¹³C isotopic signatures in this study were analyzed in relation to ¹³C isotopic abundances in the international standards VPDB (Vienna Pee Dee Belemnite; Brand et al., 2009; Wendeberg et al., 2011; JRAS scale Ghosh et al., 2005; Wendeberg et al., 2011). The precision in the laboratory of 0.012‰ for δ¹³C (for more detailed information about the laboratory analysis see Werner et al. (2001)), the application of a hyperbolic deadband (hyperbolic relaxed eddy accumulation, HREA, Bowling et al., 1999b) and comprehensive REA system and component laboratory tests made possible the resolution of up- and downdraft isotope ratio and mixing ratio differences, and consequently the determination of δ¹³C isofluxes (Wichura, 2009; Ruppert et al., 2012).

Besides the already mentioned leakage test, the balloon bag intermediate reservoirs were tested for sample contamination resulting from fractionation processes and chemical

compounds degassing from the inner balloon surface. The results proved the suitability of the balloons for a sample storage time of up to 2 h, although 30-40 minutes are enough for REA sampling (Ruppert et al., 2012). During the system test the REA device operated as in a real field experiment, but sampled standardized air from a compressed air tank. According to a previous system test in 2003 (19 samples, standard deviation: 0.014‰; Ruppert, 2008), the accuracy of the system for $\delta^{13}\text{C}$ could be maintained after the restarting in 2012 (10 Samples 0.011‰; Ruppert et al., 2012). Hence, the precision of the applied sampling operations was close to that of the mass spectrometer. The mean up- and downdraft isotope ratio difference accounted for $0.15 \pm 0.04\text{‰}$ and was larger than the instrument precision by a factor of 13. Consequently, the measurement precision accounted for only 8% and the up- and downdraft difference could be resolved very well.

3.5 Isoflux and partitioning

With adequate resolution of CO_2 and $\delta^{13}\text{C}$ -isotope mixing ratio differences in up- and downdrafts, $\delta^{13}\text{C}$ isofluxes (F_{ISO}) can be derived by introducing the δ -notation¹ to Eq. (1) (Bowling et al., 1999a; Ruppert, 2008; Wichura, 2009) and rewriting as:

$$F_{ISO} = b(H_{eff}) \sigma_w \rho_a \left(\overline{\delta^{13}\text{C}_{\uparrow} C_{\uparrow}} - \overline{\delta^{13}\text{C}_{\downarrow} C_{\downarrow}} \right). \quad (6)$$

$\overline{C_{\uparrow}}$ and $\overline{C_{\downarrow}}$ represent the CO_2 mixing ratios, and $\overline{\delta^{13}\text{C}_{\uparrow}}$ and $\overline{\delta^{13}\text{C}_{\downarrow}}$ the isotope mixing ratios of up- and downdrafts. $b(H_{eff})$ was determined by applying H to Eq. (3) and by using effectively measured CO_2 REA and eddy fluxes. σ_w was derived from the time series of the vertical wind velocity, measured by the ultra sonic anemometer. Besides F_{ISO} , information about the isotopic ratios of assimilated and respired CO_2 is also necessary for CO_2 flux partitioning based on isotopic signatures. δ_A , the isotopic ratio of assimilated CO_2 , was derived by subtracting the ecosystem discrimination of ^{13}C (Δ_e) from the isotopic ratio of the CO_2 , leaving the respective air column (i.e., the signature of the air between ground surface and REA sample inlet) that is affected by the assimilating biosphere

¹ The isotope ratios are expressed as isotopic signatures in δ -notation. All isotopic signatures are reported relative to ^{13}C isotopic abundances in the international standards VPDB (see 3.4).

(Wichura, 2009). Equation (7) is based on the mass balance equations of Lloyd et al. (1996; see also Bowling et al., 2001 and Bowling et al., 2003) and represents the tool to determine δ_A that can not be measured directly:

$$\delta_A = \overline{\delta^{13}C_{\uparrow}} - \Delta_e \quad (7)$$

Equation (8) is based on the general definition of isotopic discrimination Δ by Farquhar et al. (1989). In Buchmann et al. (1997, see also Buchmann et al., 1998), this definition was utilized to derive the ecosystem discrimination Δ_e . Ruppert (2008) found that on small timescales Δ_e can be defined as the ecosystem discrimination of the atmospheric exchange at measurement height against isotopes in the lower boundary layer air:

$$\Delta_e = \frac{\overline{\delta^{13}C_{\downarrow}} - \delta_c}{1 + \delta_c} \quad (8)$$

$\overline{\delta^{13}C_{\downarrow}}$ thereby represents the source air from above the measurement height. δ_c is the signature of the turbulent exchange which can be measured directly (Ruppert, 2008):

$$\delta_c = \frac{\overline{\delta^{13}C_{\uparrow}} \overline{C_{\uparrow}} - \overline{\delta^{13}C_{\downarrow}} \overline{C_{\downarrow}}}{(\overline{C_{\uparrow}} - \overline{C_{\downarrow}})} \quad (9)$$

δ_R , the isotopic ratio of respired CO_2 , was determined by the Keeling plot method (intercept of a plot of $\delta^{13}C$ vs. inverse carbon concentration of respiration samples; Keeling, 1958). Those samples were taken with the static alkali (NaOH) absorption method (Lundegardh, 1921; Kirita, 1971; Singh and Gupta, 1977). Dark chambers avoided assimilation and released CO_2 was captured in the solution as sodium carbonate for laboratory analysis. This method allows determination of concentration as well as isotopic ratio during night and daytime. It is well-known and often discussed that chamber methods cannot completely reproduce influences of atmospheric turbulence on the flux (Kimball and Lemon, 1971; Pumpanen et al., 2004; Rochette and Hutchinson, 2005), but alternative nighttime isotopic measurements with atmospheric methods (REA, laser techniques) for determining δ_R have a similar problem with different turbulence regimes, coming along with different atmospheric stratification, at night and daytime (Bowling et al., 2001).

Knowing F_{ISO} , δ_A and δ_R , F_{EC} could be partitioned into assimilation

$$F_A = \frac{F_{ISO} - \delta_R F_{EC}}{\delta_A - \delta_R} \quad (10)$$

and respiration flux

$$F_R = \frac{F_{ISO} - \delta_A F_{EC}}{\delta_R - \delta_A} \quad (11)$$

in accordance to Lloyd et al. (1996), Bowling et al. (2001), Bowling et al. (2003a), Ruppert (2008) and Wichura (2009). Both equations were derived from the CO₂ mass balance of a defined air column between ground surface and measurement height, considering CO₂ entering and leaving the column, CO₂ gain by respiration and loss by assimilation. By assuming adequate turbulent mixing and stationary conditions, after introduction of the corresponding CO₂ isotope ratios to the mass balance elements and after mathematical conversion, F_A and F_R can be calculated. In order to finally evaluate the quality of the partitioning tool based on isotopic signatures, results were compared with a common flux partitioning model (FPM) based on Lloyd-Taylor (Lloyd and Taylor, 1994) and Michaelis-Menten functions (Michaelis and Menten, 1913).

4 Results and discussion

4.1 Simulation of REA on managed grassland

To measure isofluxes of ¹³CO₂, the CO₂ mixing ratio is naturally the preferred proxy scalar used to control the sampling process and to determine hyperbolic deadband (H) and proportionality factor b . Because there is consistent distribution of relevant values in the different quadrants, and in order that the hyperbolic thresholds do not lead to the exclusion of too much data, $H = 1$ was chosen in accordance to Eq. (4) after simulation (cf. Ruppert et al., 2006b). Correct REA fluxes require correct b -factors. By investigating managed grassland, influences of mowing and rowen on the seasonal cycle have to be considered. Ongoing EC measurements provided data for REA simulation before and after cutting events. Days with similar weather conditions up to ten days before and twenty days after the management were used to compute $b(H)$ – on the basis of a hyperbolic deadband – by day. Those periods showed completely different results (Fig. 2). Before the management, mean $b(H)$ was 0.2, with an interquartile variation of about 20% (with exception of the

early morning and evening hours). After the management, $b(H)$ was found to have been reduced by half, but the variation had increased by more than 80% (Fig. 2b). While the mixing ratio differences of up- and downdrafts used in Eq. (3) remain negative (C sink) in almost all cases, including after the management, the EC derived CO₂ flux represented a higher NEE, which ranged up to positive and respiration dominated values. Both, the reduction and the larger variations of $b(H)$ can be attributed to the management-induced source/sink changes of the proxy scalar CO₂.

However, determination of $b(H)$ with T_S and H₂O as proxy scalars seems to be less influenced by management events (not shown in this study), but those can lack required scalar similarity to ¹³C as scalars of interest. This is an essential precondition for high quality REA measurements and must be controlled with adequate effort. In this study on all days of simulation scalar similarity between CO₂ and H₂O and T_S was evaluated by calculating scalar correlation coefficients (Eq. 5). For both combinations (CO₂ and T_S, CO₂ and H₂O), Figure 3 demonstrates an abrupt decrease of the correlation after the management. Thus, neither T_S nor H₂O are suitable alternatives to CO₂ shortly after management. Figure 3 also indicates faster recovery of scalar similarity after autumn rowen (dark symbols) than after mid summer mowing (bright symbols). This can be linked to greater intervention in the ecosystem in mid summer, i.e. removing more productive biomass than in autumn. In both cases scalar similarity increased with ecosystem recovery up to pre-cutting values. The lack of scalar similarity after the management confirms dependence on plant physiology (Williams et al., 2007) and source-sink influences (Andreas et al., 1998a; Katul et al., 1999; Katul and Hsieh, 1999; Ruppert et al., 2006b; Held et al., 2008; Ruppert, 2008). In general, it is suggested that REA not be applied shortly after management events due to the fact that b can only be properly determined before management events and after an adequate period of ecosystem recovery. Under the environmental conditions present in this study, it is suggested that REA not be applied for 22 days after the summer mowing and for 12 days after the rowen in autumn (Fig. 3). Present diurnal variations of $b(H)$ advise against application of constant b factors.

4.2 REA measurements

REA measurements in this study were conducted prior to, and five weeks after, summer mowing to fulfill the precondition of an undisturbed ecosystem. Apart from the selection according to the meadow management, two REA measurement days with different wind conditions were chosen. With an average of 2 m s^{-1} , the wind velocity (u) on 22 June 2010 was half as large as on 25 August 2010 (on average 4 m s^{-1} during the measurement period). On both days mean air temperature (T) and mean incoming shortwave radiation (K_{in}) were comparable to some extent. F_{ISO} as well as $\delta^{13}\text{C}$ values partly follow fluctuations of K_{in} , although REA sampling processes lasted 40 minutes to generate adequate amounts of sample air, and unfortunately integrated over very different radiation conditions (Fig. 4). First, the enrichment of ^{13}C in the atmosphere can be observed in Figure 4e. Sufficient water availability due to a high ground water level and moderate air temperature ($\leq 17 \text{ }^\circ\text{C}$) excludes reduced stomatal conductance, i.e. noon depression. High enrichment of ^{13}C at noon on 22 June relies on increased assimilation. This assumption is supported by the development of the NEE that shows the largest C uptake during that time (Fig. 5a). The pattern of F_{ISO} acts to a certain extent in accordance with the differences of the $\delta^{13}\text{C}$ values of up- and downdrafts. On both days the ranges of F_{ISO} match results of other studies (Bowling et al., 2001; Wichura, 2009). This also applies to the evening break-down of F_{ISO} due to missing up- and downdraft isotope ratio differences, coming along with absent shortwave radiation and consequently biosphere activity (last sampling on 22 June). With the exception of this last measurement, adequate $\delta^{13}\text{C}$ differences between up- and downdraft samples were always achieved (on average $0.15 \pm 0.04\text{‰}$; precision of IRMS Jena: 0.012‰ , Werner et al., 2001). In addition to its dependence on wind velocity, i.e. σ_w in Eq. (6), factor b is decisive for F_{ISO} . Therefore $b(H_{\text{eff}})$ was calculated from directly measured REA up- and downdraft samples and appropriate EC fluxes. In contrast to simulated $b(H)$, effective b -factors $b(H_{\text{eff}})$ do not overestimate the CO_2 mixing ratio differences (i.e. underestimate the necessary size of b), due to a certain inevitable imprecision of the physical sample separation process of the measurement system compared to the simulation. Sample carry-over during the real REA measurement can also be a reason for that. Thus, effective b -factors were slightly higher (0.28 ± 0.05) than

the simulated values shown in Figure 2. This has already been observed in previous studies (Baker et al., 1992; Beverland et al., 1996; Moncrieff et al., 1998; Ruppert et al., 2012).

4.3 Flux partitioning

To partition NEE into assimilation and respiration fluxes based on the isotopic method, their isotopic signatures δ_A and δ_R , as well as F_{ISO} and F_{EC} , are required (Eqs. 10 and 11). δ_A and δ_R are sensitive factors in the model that have to be discussed in detail (Ogée et al., 2004; Ruppert, 2008). Determination of δ_R is based on the Keeling plot method (Keeling, 1958). Therefore, samples were taken with the static alkali absorption method in dark soil chambers. The complex assignment of δ_R values to temporally varying photosynthetic activity due to time lag effects (Knohl and Buchmann, 2005), and unsolved problems applying night-time δ_R measurements, suggest the application of integrative static chamber measurements. However, it is possible to improve resolution of the δ_R data with modern laser $\delta^{13}C$ measurements involving considerable expense (Griffis et al., 2004; Bowling et al., 2003b). Independent of the kind of data acquisition, the sensitivity of δ_R related to $\delta^{13}C$ measurements has to be evaluated (Zobitz et al., 2006; Pataki, 2003). In this study the Keeling plot intercept accounted for -24.9‰ (Fig. 6) with a standard error of 1.7‰ , within a 95% confidence interval of $\pm 4.3\text{‰}$.

On 22 June and 25 August in 2010, before and long after meadow mowing, all parameters were determined in order to partition NEE into assimilation (F_A) and respiration (F_R ; Fig. 5) based on two different approaches: the common flux partitioning model (FPM), based on Lloyd Taylor and Michaelis Menten functions, and the isotopic flux partitioning approach (Eqs. 10 and 11). On both days NEE (Fig. 5) shows variations according to incoming shortwave radiation (Fig. 4), with maximum CO_2 sink capacity of almost $0.02 \text{ mmol m}^{-2} \text{ s}^{-1}$ during the day. While the morning rise of photosynthetic activity was not sampled, evening breakdown to a respiration-dominated system was captured. The last value in Figure 5a in the evening shows a special case. There is no longer any difference between up- and downdraft isotope ratios, so that isoflux, and consequently assimilation and respiration fluxes, become zero. This comes along with a lack of photosynthesis and discrimination, but above all with turbulent fluxes that come to a standstill, as confirmed

by a very small NEE (Fig. 5) and low wind velocity and incoming shortwave radiation (Fig. 4). This pattern is not shown by the Lloyd Taylor and Michaelis Menten function based FPM. Apart from that, the isotopic flux partitioning shows a much greater variability, whereas the FPM insufficiently reproduces natural respiration changes, causing assimilation fluxes to exactly follow the NEE. Sometimes both approaches provide partially similar fluxes, but the isotopic model is able to describe various underlying fluxes of the NEE; that is, more intense reactions to environmental conditions are attributed to the ecosystem (Ruppert, 2008). The quite constant respiration provided by the temperature-based FPM results from relatively small temperature variations during both periods around the measurement days. Temperature is only one of the driving mechanisms of respiration; for example, photosynthetic activity supplies root exudates to soil life and accounts for a large portion of root-derived respiration (Kuzyakov and Gavrichkova, 2010). Discrimination of ^{13}C is an input factor in the isotopic model. It is directly coupled to all assimilation-based processes. These become apparent in the assimilation flux closely connected to the incoming shortwave radiation. The same applies to wind velocity, essential for atmospheric fluxes and considered only in the isotopic model as an input parameter of F_{ISO} . The diurnal cycle of the assimilation flux – determined from F_{ISO} – (Fig. 5) can be explained clearly by the diurnal cycles of incoming shortwave radiation and wind velocity (Fig. 4). Especially incoming shortwave radiation drives surface temperature and assimilation dependent, soil organic matter derived respiration (Kuzyakov and Gavrichkova, 2010). Almost all values of the isotopic model show these dependences to some extent. This representation of environmental influences in combination with the accordance to the established common flux partitioning model suggests good performance of the isotopic model, and there is no evidence for comparable restrictions found for complicated coupling regimes in high vegetation ecosystems (Ruppert, 2008; Wichura, 2009).

Conclusions

Detailed investigation of pre- and post mowing conditions by REA-simulations on managed grassland demonstrated serious constraints for REA-application directly after management. At this time, simulated b -factors showed larger uncertainty and decreased

strongly because the mixing ratio differences in the simulation did not follow the NEE determined by EC to have positive fluxes. Also, the scalar similarity assumption was not fulfilled for the evaluated proxy scalars CO₂, T and H₂O after management. Consequently, REA technique cannot be applied shortly after meadow management without the risk of huge REA-flux errors. This restriction should be carefully considered in future REA-studies. A distinct decision of when to use REA again depends on environmental conditions and plant community structure. Both factors are decisive for plant community recovery and hence the development of scalar concentration and flux behavior. For several scalar quantities plant physiology monitoring and consideration of the state of the ecosystem recovery could be helpful. This study suggests waiting at least 22 days in summer and 12 days in autumn after management in like circumstances. With carefully evaluated *b*-factors, application of a hyperbolic deadband and high precision laboratory analysis, up- and downdraft differences can be resolved and isofluxes can be derived.

The NEE was partitioned by an isotopic modeling approach based on information about isotopic ratios of assimilation and respiration fluxes, EC- and isoflux, respectively. It turned out that the isotopic approach works well on the grassland experiment site compared to former studies where it was applied over forest ecosystems with special coupling regimes. Moreover, it can enhance results of a common flux partitioning tool based on Lloyd-Taylor and Michaelis Menten functions. An advantage is a better reproduction of environmental conditions, due to directly including ecosystem discrimination of ¹³C and wind velocity into the model. However, the method is very sensitive and requires exact determination of the isotopic signatures (Ruppert, 2008). Also given uncertainties regarding determination of respiration characteristics have to be further investigated. Chamber measurements require detailed consideration of atmospheric conditions (Riederer et al., 2013).

REA application in general is expensive and time consuming and is therefore only applicable for short term and special investigations. Its versatility and the information about NEE component flux variability gained through its use justify its application in ecosystem sciences. However, in the future it will be more and more replaced by e.g. direct isotopic eddy covariance measurements that are also adapted for long term experiments (Wehr et al., 2013).

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Figures

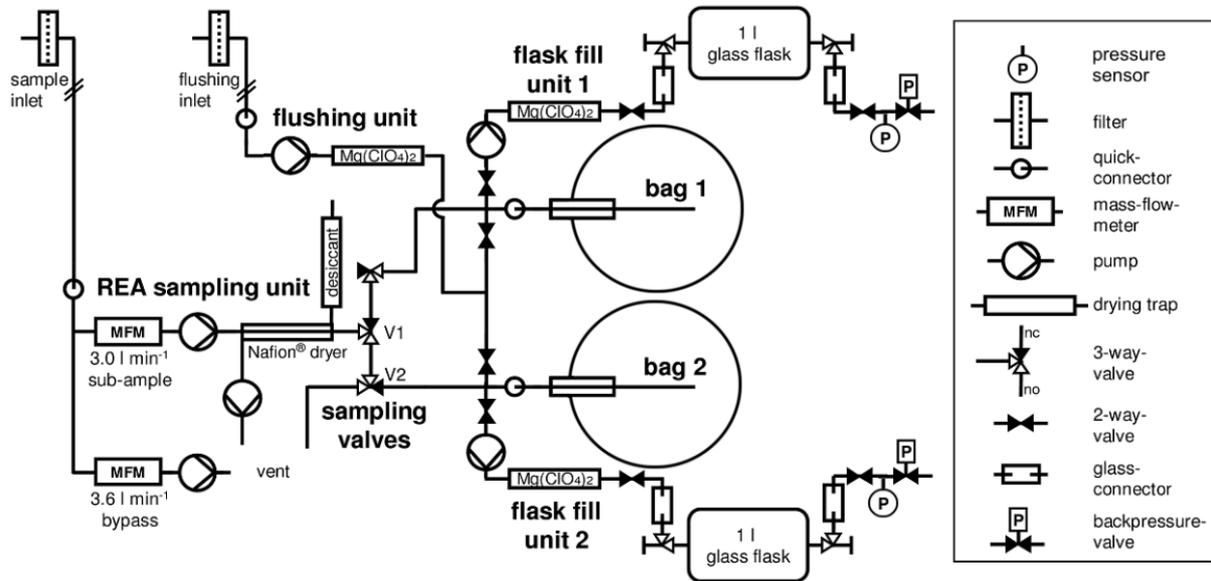


Figure 1. Design of the whole-air REA system (Ruppert et al., 2012)

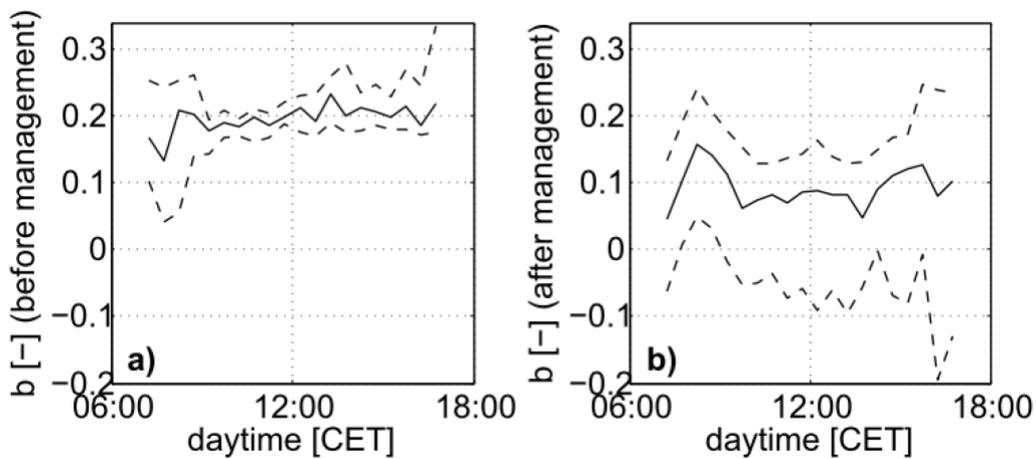


Figure 2. Daytime $b(H)$ before (a) and after cutting events (b); data from rowen in 2009, mowing and rowen in 2010 and mowing in 2011, median (solid line), 25% and 75% quantile (dashed line) of 16 days before and of 27 days after management are illustrated, $H = 1.0$, (proxy) scalar = CO_2 .

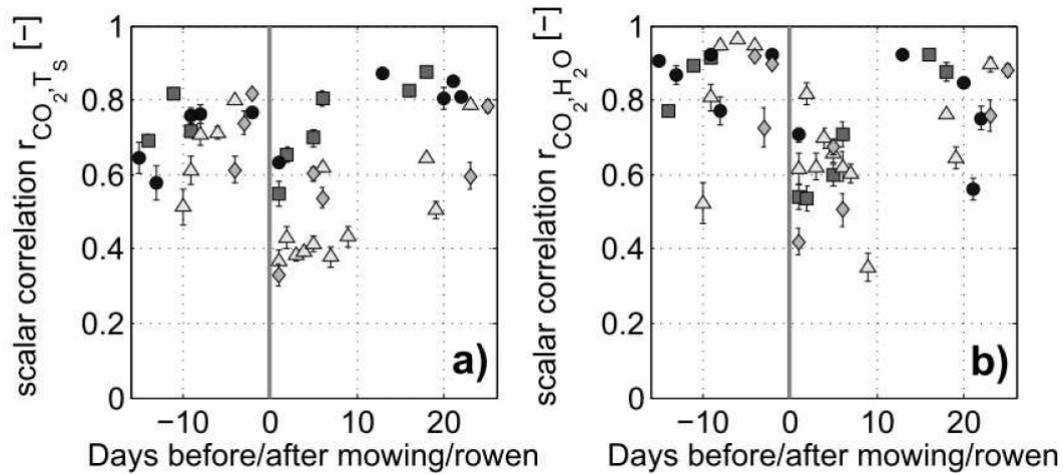


Figure 3. Absolute scalar correlation coefficients between CO_2 and sonic temperature T_s (a) and CO_2 and water vapor H_2O (b). Negative x-axis values indicate days before, positive values days after mowing and rowen, respectively. Dark squares represent rowen data in September 2009, bright upward triangles mowing in July 2010, black circles rowen in September 2010 and grey diamonds the late mowing in August 2011. The error bars show standard errors of the daily mean of r between 09:00 and 17:00 CET.

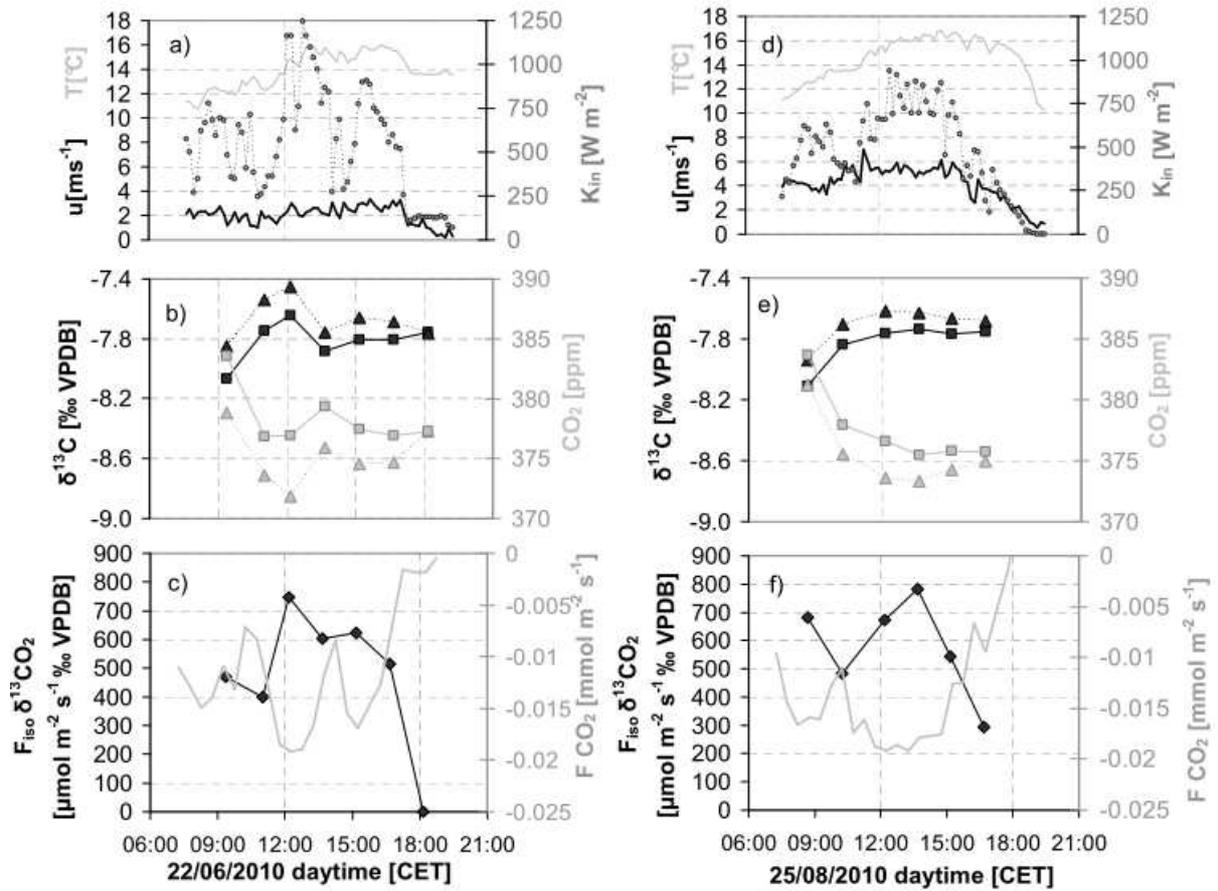


Figure 4. Incoming shortwave radiation K_{in} (grey dashed line with circles), temperature T and wind velocity u (bold black line) are illustrated in a) and d); dark upward triangles in b) and e) represent isotopic composition of updraft, dark squares of downdraft CO_2 , bright symbols represent CO_2 mixing ratios; c) and f) show the $\delta^{13}\text{CO}_2$ isoflux (F_{iso} , dark diamonds, set in the middle of the 40 minute measurement interval; bright lines show the CO_2 flux.

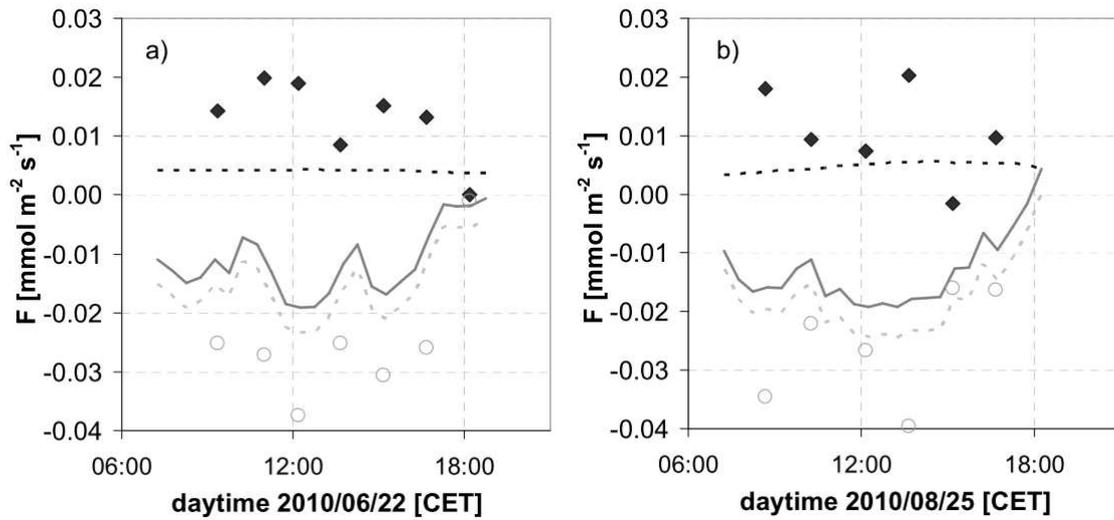


Figure 5. Comparison of NEE flux partitioning with isotopic background (respiration flux F_R : black diamonds, assimilation flux F_A : light grey circles) and a common FPM (dashed lines in same colors); the NEE measured by eddy-covariance is illustrated as dark grey solid line.

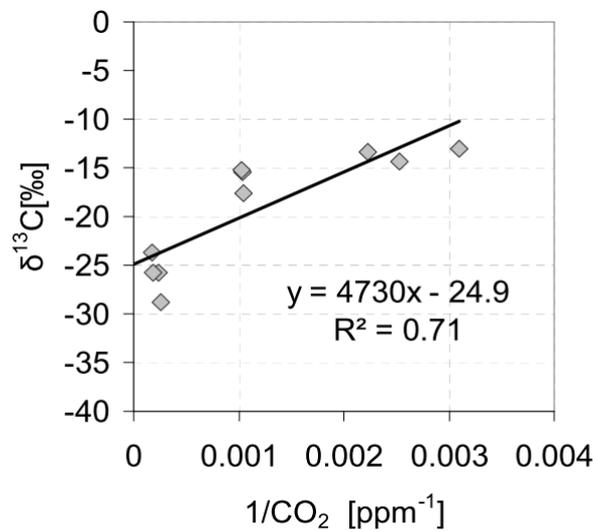


Figure 6. Keeling plot of respiration samples for determination of isotopic ratio of respiration; δ_R ; Keeling plot intercept: -24.9 with a standard error of 1.7‰, within a 95% confidence interval of $\pm 4.3\%$. The solid line indicates the orthogonal regression line. R = correlation coefficient.

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Net ecosystem CO₂ exchange measurements by the closed chamber method and the eddy covariance technique and their dependence on atmospheric conditions – A case study

M. Riederer^{1,*}, A. Serafimovich^{1,}, T. Foken^{1,2}**

[1]{Department of Micrometeorology, University of Bayreuth, D–95440 Bayreuth, Germany}

[2]{Member of Bayreuth Center of Ecology and Environmental Research (BayCEER), University of Bayreuth, D–95440 Bayreuth, Germany}

[*] {now: Regensburg Center of Energy and Resources, Regensburg University of Applied Sciences, D–93049 Regensburg, Germany}

[**] {now: German Research Centre for Geosciences, Helmholtz Centre, D–14473 Potsdam, Germany}

Correspondence to: M. Riederer (michael.riederer@uni-bayreuth.de)

T. Foken (thomas.foken@uni-bayreuth.de)

Abstract

Carbon dioxide flux measurements in ecosystem sciences are mostly conducted by eddy covariance technique or the closed chamber method. But there is a lack of detailed comparisons that assess present differences and uncertainties. To determine underlying processes, a ten-day, side-by-side measurement of the net ecosystem exchange with both techniques was evaluated with regard to various atmospheric conditions during the diurnal

cycle. It was found that, depending on the particular atmospheric condition, the chamber carbon dioxide flux was either: (i) equal to the carbon dioxide flux measured by the reference method eddy covariance, by day with well developed atmospheric turbulence, (ii) higher, in the afternoon in times of oasis effect, (iii) lower, predominantly at night while large coherent structure fluxes or high wind velocities prevailed, or, (iv) showed less variation in the flux pattern, at night while stable stratification was present. At night – when respiration forms the net ecosystem exchange – lower chamber carbon dioxide fluxes were found. In the afternoon – when the ecosystem is still a net carbon sink – the carbon dioxide fluxes measured by the chamber prevailed. These two complementary aspects resulted in an overestimation of the ecosystem sink capacity by the chamber of 40 % in this study.

1 Introduction

Net ecosystem exchange (NEE) of grasslands is today predominantly determined by eddy covariance (EC) technique (Moncrieff et al., 1997; Baldocchi, 2003; Foken et al., 2012a; Wohlfahrt et al., 2012) and the chamber method (Davidson et al., 2002; Subke and Tenhunen, 2004; Denmead, 2008). The chamber method also becomes relevant when measuring underlying fluxes of NEE (e.g. ecosystem respiration, R_{ECO}) directly and separately. Also gross primary production (GPP) of the biosphere can be easily determined by combining the use of dark (R_{ECO}) and transparent chambers (NEE) and simple subtraction of the resulting fluxes.

Numerous comparison experiments between different chambers (Pumpanen et al., 2004; Rochette and Hutchinson, 2005) and between chamber– and EC–data (Subke and Tenhunen, 2004; Kutzbach et al., 2007; Myklebust et al., 2008, Wang et al., 2013) can be found in the literature. Comparisons between chamber and EC–measurements are also available for other trace gases, for example Werle and Kormann (2001) found that chambers may overestimate CH_4 emissions by up to 60–80%. Differences were for example found due to methodological problems under high vegetation (Subke and Tenhunen, 2004), at times with low turbulence intensity (van Gorsel et al., 2007), at night over complex surfaces (Myklebust et al., 2008), due to poor regression analysis in the chamber software (Kutzbach et al., 2007) or different target areas (Reth et al., 2005). The

EC method is, by definition, a direct measuring method (Montgomery, 1948; Obukhov, 1951; Swinbank, 1951) for determining turbulent fluxes. However, several conditions must be fulfilled before the method can be applied as a reference method. Most important in this context are steady state conditions, flat and homogeneous terrain and turbulent exchange conditions (Lee et al., 2004; Foken 2008; Aubinet et al., 2012). The control of these conditions is achieved by applying data quality tools (Foken and Wichura, 1996; Vickers and Mahrt, 1997; Foken et al., 2004), the application of which has recently come to represent the state of the art. In contrast to EC – that measures an integrated signal from a large flux footprint area (Rannik et al., 2012) – it is often challenging to achieve adequate representativeness with the chamber method on ecosystem scales (Reth et al., 2005; Laine et al., 2006; Denmead, 2008; Fox et al., 2008). In any case, both EC and chamber methods must be reviewed for inaccuracies (Davidson et al., 2002), and due to the fact that real fluxes are always unknown under field conditions, it is impossible to validate flux measurements by any technique (Rochette and Hutchinson, 2005).

Chamber measurement technique has improved during recent years and eliminated many chamber effects (Rochette and Hutchinson, 2005) to the point where pressure inconsistencies between in- and outside the chamber at various wind velocities can be avoided (Xu et al., 2006). But some challenges still remain, for example inside chambers, atmospheric turbulence cannot be reproduced (Kimball and Lemon, 1971; Pumpanen et al., 2004; Rochette and Hutchinson, 2005) even when ventilators are used for mixing (Kimball and Lemon, 1972).

Atmospheric turbulence has a typical size spectrum and distribution of the turbulent eddies, depending on height and surface structure. In particular, larger, low-frequency flow patterns, i.e. coherent structures (Collineau and Brunet, 1993; Gao et al., 1989; Thomas and Foken, 2007), may cause differences between chamber and EC measurement results. Another cause of flux differences can be differing atmospheric stratification. Closed chambers completely cover the ecosystem during the measurement process and thereby alter the natural long wave radiation balance to almost zero. This causes reduced surface cooling, weak development of stable stratification and finally higher fluxes compared to EC.

In this study it is not the differences in NEE between two measurement principles in general, but rather the changing differences under varying atmospheric conditions in the course of the diurnal cycle, which is investigated.

2 Material and Methods

2.1 Study area

The comparison experiment was conducted from May 25th to June 3rd in 2011 on an extensively managed submontane grassland site at the edge of the low mountain range “Fichtelgebirge” in northeast Bavaria, Germany. The site is located on flat terrain 624 m a.s.l. (50°05′25″N, 11°51′25″E) between the “Großer Waldstein” (elevation: 877 m) to the north and the “Schneeberg” (1051 m) to the south. Thus, a channeled wind field in west–east direction with west (263°) as prevailing wind direction is created at the site. Most of the data were collected under ideal weather conditions without rainfall and with sufficient global radiation. Weak data due to dewfall on the instruments and one heavy rainfall event (38.2 mm) in the night of May 31st to June 1st were excluded. The canopy height was about 20 cm. Thus, the chamber could be installed without any cutting of the vegetation.

2.2 Eddy covariance

For the determination of the CO₂ flux, the concentration was measured by an open–path gas analyzer (LI–7500, LI–COR Biosciences, Lincoln, Nebraska USA), and the wind vector by a 3D sonic anemometer (CSAT3, Campbell Scientific, Inc., Logan, UT USA) at high frequency (20 Hz), 2.5 m above ground. Data were stored on a data logger (CR3000, Campbell Scientific, Inc., Logan, UT USA) and collected daily by a computer system as a backup. Data were post processed and quality controlled based on latest micrometeorological standards by the software package TK2, developed at the University of Bayreuth (Mauder and Foken, 2004). This still evolving software (TK3 has become available in the meantime: Mauder and Foken, 2011) incorporates all necessary data correction and data quality tools (Foken et al., 2012b). It was successfully proved in comparison with six other commonly used software packages (Mauder et al., 2008). For

every averaging interval of 30 minutes the included quality flagging system evaluated stationarity and turbulence and marked the resulting flux with quality flags from 1 (very good quality) to 9 (very low quality) (Foken and Wichura, 1996; Foken et al., 2004). In this study only data with quality 3 or better were used. Also footprint analysis (not shown here) after (Göckede et al., 2004; Göckede et al., 2006; Rannik et al., 2000) was performed to assure that the measured data exclusively represented the target land use type grassland, i.e. the ecosystem measured by the chamber (cf. Reth et al., 2005). Due to the channeled wind regime, two club-shaped footprints evolved in the western and eastern directions. Thus, disturbances of the turbulence measurements could be easily avoided by installing all other experimental devices close to the EC mast, but perpendicular to the main wind direction. Accompanying measurements of important micrometeorological parameters such as up- and downwelling short- and long wave radiation, air and soil temperature, humidity and soil moisture and precipitation were accomplished by an automated weather station and stored as 10-minute averages.

2.3 Chamber system

The applied system (LI-8100-104C, transparent for NEE measurements at low vegetation, LI-COR Biosciences, Lincoln, Nebraska USA) was an automated flow-through non-steady-state soil chamber, where sample air was constantly circulated between the chamber and an infrared gas analyzer (IRGA) by a rotary pump with 1.5 L min^{-1} through a chamber volume of 4822 cm^3 . The CO_2 flux was estimated from the rate of CO_2 concentration change inside the chamber during a close time of 90 seconds. The chamber was designed to minimize perturbations to the surrounding environmental conditions. For example the base plate was perforated to avoid heating of the surface and a concentration gradient-induced impedance of soil respiration (LI-COR, 2004). The soil collars which included an area of 318 cm^2 were pre-installed 10 cm deep in the soil two weeks before the experiment to create a perfect seal and to avoid disturbances of the CO_2 efflux by cut and wounded plant roots at the beginning of the measurement period. Due to the channeled wind field on the site (see section 2.1), the chamber could be installed very close to the eddy covariance mast without disturbing the flux footprint. The chamber had a lift-and-rotate drive mechanism that rotated the bowl-shaped chamber 180° away from the collar.

This shape allowed good mixing by means of the circulation of the sample air through the IRGA alone, without a ventilator (LI-COR, 2004). Barometric- and – above all – turbulence-induced pressure fluctuations above the ground surface influence the efflux from the soil. Thus, modern chambers are equipped with a venting tube that transmits atmospheric pressure changes to the chamber headspace (Rochette and Hutchinson, 2005). LI-COR installed a patent-pending pressure vent with tapered cross section at the top of the chamber, that minimizes pressure pulses at chamber closing and allows the tracking of ambient pressure under calm and windy conditions by eliminating the Venturi effect (Conen and Smith, 1998) occurring at former simple open vent tubes (Xu et al., 2006). The exchange through the venting tube is negligible compared to the CO₂ diluting effect by water vapor during the measurement which in turn is corrected by the measurement software (LI-COR, 2004). For R_{ECO} measurements a dark chamber is used that avoids CO₂ uptake by assimilation. NEE is measured by a chamber with a transparent dome that enables CO₂ uptake by assimilation as well as respiration processes inside. The transparent chamber for the NEE comparison was closed for 90 seconds four times during a half-hour period. In the meantime the system was flushed for 135 seconds, the dark chamber was measuring for 90 seconds (data were required for another study and not used in this one) and the system was flushed with ambient air again. The closing and opening process of the transparent chamber as part of the flushing time lasted 13 seconds each.

2.4 Typical exchange conditions

The application of the eddy covariance technique requires turbulent conditions (Foken et al., 2012a). Ecologists often evaluate this using a friction velocity threshold (Goulden et al., 1996) but more precise is a test on steady-state conditions and the fulfillment of typical similarity conditions (Foken and Wichura, 1996). At daytime in most cases both criteria are fulfilled whereas nighttime exchange conditions are more challenging.

Already in the late afternoon stable stratification of the near surface air layer begins with cooling due to evaporation and the long wave upwelling radiation outbalancing the long wave downwelling radiation. Exchange is poor under stable conditions and, for example, the respiration causes the carbon dioxide concentration to increase in the first centimeters of the atmosphere up to a partial pressure equivalent to that in the soil, which consequently

reduces the gas exchange. However, an ecosystem covered with a chamber dome is subjected to balanced outgoing and incoming long wave radiation and therefore less cooling at that time of the day. Naturally under those conditions the so called oasis effect occurs, which is named after the moisture-dependent cooling effect occurring in oases and which is defined as a sensible heat flux (Q_H) changing to negative values in combination with a still large positive latent heat flux (Q_E) and solar radiation (Stull, 1988; Foken, 2008). A lack of sensible heat causes reduction of buoyancy and consequently turbulence. This is directly detected by the EC technique, i.e. exactly the measurement of turbulent fluxes (Aubinet et al., 2012). In addition to the radiation effect, the reaction of the chamber system is also less pronounced due to the physical barrier to the surrounding, increasingly stable stratified, air masses. With the sunset the remaining assimilation potential is gone, the difference between both systems declines, and other processes come to the fore.

Under stable stratification and low turbulence the flux-contribution of coherent structures to the entire flux increases (Collineau and Brunet, 1993; Gao et al., 1989; Thomas and Foken, 2007; Holmes et al., 2012). These well-organized structures, with typical periods of 10–100 s, are caused by strong roughness or landscape heterogeneities such as tree lines, bushes and ditches. Coherent structures in a steady state can be measured by eddy covariance technique (Desjardins, 1977). Analyzing methods for coherent structures are based on, for example, wavelet technology and were presented by Collineau and Brunet (1993), Thomas and Foken (2005) and Serafimovich et al. (2011). In the present study, we applied the method described by Thomas and Foken (2005) to determine the flux by coherent structures (F_{CS}) and its contribution to the entire flux ($F_{CS} F_{ent}^{-1}$).

3 Results and discussion

Scatter charts are often utilized in literature when measurement technique comparisons are discussed. However, they provide only a first impression of the overall behavior of both systems, and in this study Figure 1 is intended as an introduction to further detailed breakdown of the behavior into underlying processes. So as not to adulterate the comparison results, data with bad quality were excluded by the quality flagging system (16 %) and no gap filling procedures were conducted. In any event, only data were used when both systems provided data of high quality. Data gaps were predominantly occurring at

night, when CO₂ source fluxes (positive sign) prevailed. Thus, the resulting mean CO₂ values of -4.0 (EC) and -5.6 μmol m⁻² s⁻¹ (chamber) for the overall 10-day balance might be overestimated. Hence, at that time, both EC and chamber define the ecosystem to be a CO₂ sink, but the absolute value of the chamber sink flux was 40 % larger than that of EC. This is similar to other studies (Wang et al., 2009; Fox et al., 2008) and includes – in our case – smaller chamber CO₂ source fluxes of 26 % during the night and larger chamber CO₂ sink fluxes of 14 % during the day (negative sign). A first indication as to the cause of the large difference at night may be provided by the kind and dimension of scattering of the measured fluxes, presented in Figure 1 as interquartile ranges. While daytime CO₂ fluxes of both techniques scatter quite similarly, with interquartile ranges of 0.0086 mmol CO₂ m⁻² s⁻¹ and 0.0094 mmol CO₂ m⁻² s⁻¹, respectively, for positive nighttime CO₂ fluxes, much larger scattering in EC data (interquartile range: 0.0039 mmol CO₂ m⁻² s⁻¹) than in chamber data (0.0018 mmol CO₂ m⁻² s⁻¹) could be recognized (see Figure 1 and cf. Janssens et al., 2001).

This kind of aggregation of the positive chamber fluxes (cf. Laine et al., 2006) had various associated reasons that are explained in the following. There must be also an explanation for the domination of the chamber in small negative CO₂ fluxes, not only when both systems showed fluxes with opposite directions (Fig.1, light grey filled circles) but also when both were negative. To investigate underlying short-term effects on the comparability, EC-chamber flux differences –normalized with the EC-flux – were calculated and illustrated as mean diurnal cycles of the whole measurement period (Fig.2a)

The characteristics of the normalized EC-chamber flux difference suggested a classification into four different periods. The early morning transition time was affected by sunrise, developing turbulence and temporary wet instruments due to dewfall, and this prevented proper data analysis for this period. Later, during the day, when the atmospheric turbulence was well developed, the mean difference was almost zero, i.e. both systems worked well and showed similar results. In contrast, in the late afternoon, CO₂ sink fluxes within the chamber were sustained longer and were larger, resulting in a flux up to twice as large as the EC flux (Fig. 2a). The reason was defined as the oasis effect, i.e. cooling and stabilization effects outside the chamber (see section 2.4). In Figure 2b just the normalized flux differences during periods of prevailing oasis effect are considered, which precisely reproduces the late- and to a small extent early afternoon chamber dominance. Nearly all

measurements influenced by the oasis effect show larger chamber fluxes (Fig. 3a). Also two thirds of the situations with contrary EC–chamber flux directions (filled circles, Fig 1 and Fig 3a) and the higher sink fluxes of the chamber at small values could be directly explained by the oasis effect (black circles, Fig 3a). With the sunset this effect disappears, as does the assimilation potential of the ecosystem, and the difference between both systems declines.

After a short evening transition time the fourth period with typical nighttime conditions arises – characterized by predominantly stable stratification (Fig. 2d) and increasing exchange by coherent structures (Fig. 2c). For mid–latitudes this is the typical diurnal cycle for stratification (Foken, 2008). Coherent structures can cause 50–100 % of the gas exchange during nighttime and 10–20 % during the day above a forest (Thomas and Foken, 2007). The influence of coherent structures might be less above meadows due to the negligible mixing layer (roughness sublayer). In contrast to daytime CO₂ fluxes that scatter quite similarly (see interquartile ranges in Figure 1), nighttime chamber fluxes scatter less than half as much as the EC fluxes: The chamber measures a virtually constant flux during the night. As Figure 3b, c and d illustrate, this predominantly occurs at times with high atmospheric stability, presenting along with low wind velocity and a cool ground surface, i.e. little outgoing long wave radiation. While the EC system responds to the smallest changes of the atmospheric conditions as well as the nighttime ecosystem respiration flux does, the chamber is directly connected to the ground surface – where the ecosystem respiration is more or less constant – with only minor influences from the surrounding atmosphere (Norman et al., 1997; Reth et al., 2005; Lai et al., 2012), transferred into the chamber system exclusively by the pressure vent (Xu et al., 2006). Besides coherent motions, which are generated by braking gravity waves or under the influence of low level jets (Karipot et al. 2008), heating due to dewfall causes slightly higher turbulent fluxes during nighttimes. The condensation heat thereby reduces the downward sensible heat flux and the strong stable stratification. Both processes are related to slightly higher wind velocities (Fig. 4b) and larger EC flux results (Fig. 1). While EC measures that wide range of CO₂ fluxes, the parameters illustrated in Figure 3b, c and d turned out to be particularly responsible for the uniformity of the chamber flux. To clarify under which conditions the EC flux is notably larger or smaller than the chamber flux, nighttime data with higher EC fluxes were compared to those that show higher chamber

fluxes. A Student's *t*-test for dependent samples indicated no differences for the flux by coherent structures (F_{CS}), z/L and I_{out} , but did so for the wind velocity u and the friction velocity u_* (Fig. 4; u_* is not presented since the result is equivalent to u).

However, EC and chamber nighttime respiration fluxes measured at high wind velocities (largest 25 %, $u > 2.9 \text{ ms}^{-1}$) are within the same range close to the bisecting line in Figure 5a but with a significant tendency to larger EC fluxes. This coincides with a study of Denmead and Reicosky (2003), who found an increase of the EC- to chamber-flux ratio with the wind velocity. Although the chamber reproduces the flux variations very well at high wind velocities, i.e., it is able to describe small as well as larger fluxes, it generally underestimates the flux. Hence, at night, in addition to the stratification effect, situations with high wind velocities result in larger EC than chamber CO_2 fluxes. But these cannot really explain the highest EC fluxes in times of uniform chamber performance. It was found that some of those situations occurred together with large coherent structure fluxes (F_{CS} , Fig. 5b). In the experiment region, coherent motions were already detected as a consequence of low-level jets reaching the ground and breaking gravity waves (Foken et al., 2012c). Coherent structures appear sporadically (average in this study: 38 h^{-1}). Thus, the total size of the coherent structure flux is less than the typical turbulent flux, yet coherent motions produce turbulence that obviously is recognized by EC, but not by the chamber technique (Fig. 5b).

4 Conclusions

Ecosystem processes are coupled to atmospheric conditions. A measurement system should be able to represent the resulting fluxes in a reasonable way. Otherwise, already small differences at small temporal scales may sum up to large errors in the estimation of the resulting flux. Because the difference between chamber and EC flux strongly depends on the diurnal variation of the atmospheric conditions, especially sporadic short term chamber measurements as well as repeated chamber measurements at specific times of day are likely to be biased.

Chamber fluxes are larger than EC fluxes in the late afternoon due to surface cooling and development of stable stratification, which in turn reduces the turbulent exchange. During

times of this oasis effect, the flux regime of the day is upheld longer in the evening within the chamber and the real atmospheric conditions are not represented.

During the night a quite uniform chamber flux and an EC flux with a much higher variability were observed. Detailed investigation of the relevant parameters revealed that the nighttime stable stratification, together with low wind velocities and low outgoing long wave radiation, support the uniformity of the chamber but not the EC flux. A greater variation of the chamber flux data was only found at times with high wind velocities and high friction velocities, respectively, which also resulted in a certain agreement with EC, but with overall higher EC fluxes. Hence, the chamber is less sensitive to atmospheric conditions that control the flux, because it is always less coupled to the surrounding atmosphere than EC (Lai et al., 2012; Dore et al., 2003; Reth et al., 2005).

Coherent structures were also expected to cause higher EC fluxes in general, but it was found that this was only the case with the very largest coherent structure fluxes. Those could explain a number of situations with larger EC fluxes.

Although at our experimental site EC provides satisfying results for the whole diurnal cycle – assuming that data quality regarding turbulence and stationarity is properly controlled – chamber flux measurements require accompanying assessment of at least wind velocity, radiation and temperature, to evaluate atmospheric conditions to some extent. Above all, during the night the strongest forcing parameters, global radiation and the CO₂ sink flux by assimilation are missing. Since the long wave radiation balance is almost zero within the chamber anyway and the night time respiration flux from the soil is more constant than the CO₂ flux during the day, there should be nothing left to trigger variations in the chamber CO₂ flux, which do, however, occur.

The positive message is that both techniques show proper and comparable results from late morning – when all instruments have dried from dewfall – until afternoon, when the oasis effect gains more and more influence.

Chamber measurement technique has made progress in the last years but its insensitivity to various atmospheric conditions suggests such micrometeorological tools as EC are preferable for the investigation of those processes and the determination of ecosystem fluxes.

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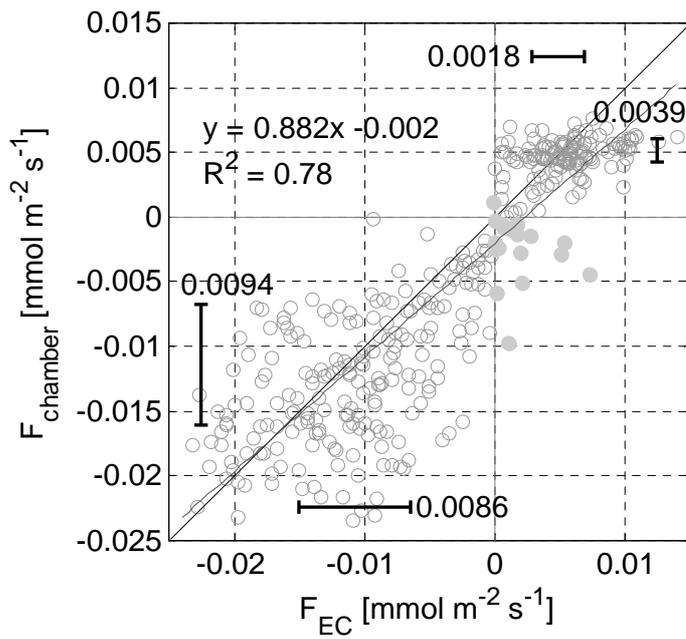


Figure 1. Scatter plot of EC- and chamber-determined NEE, light grey filled circles represent CO₂ fluxes with opposite directions, black bars show interquartile ranges of EC-/chamber CO₂ source and sink fluxes, respectively (opposite CO₂ fluxes excluded).

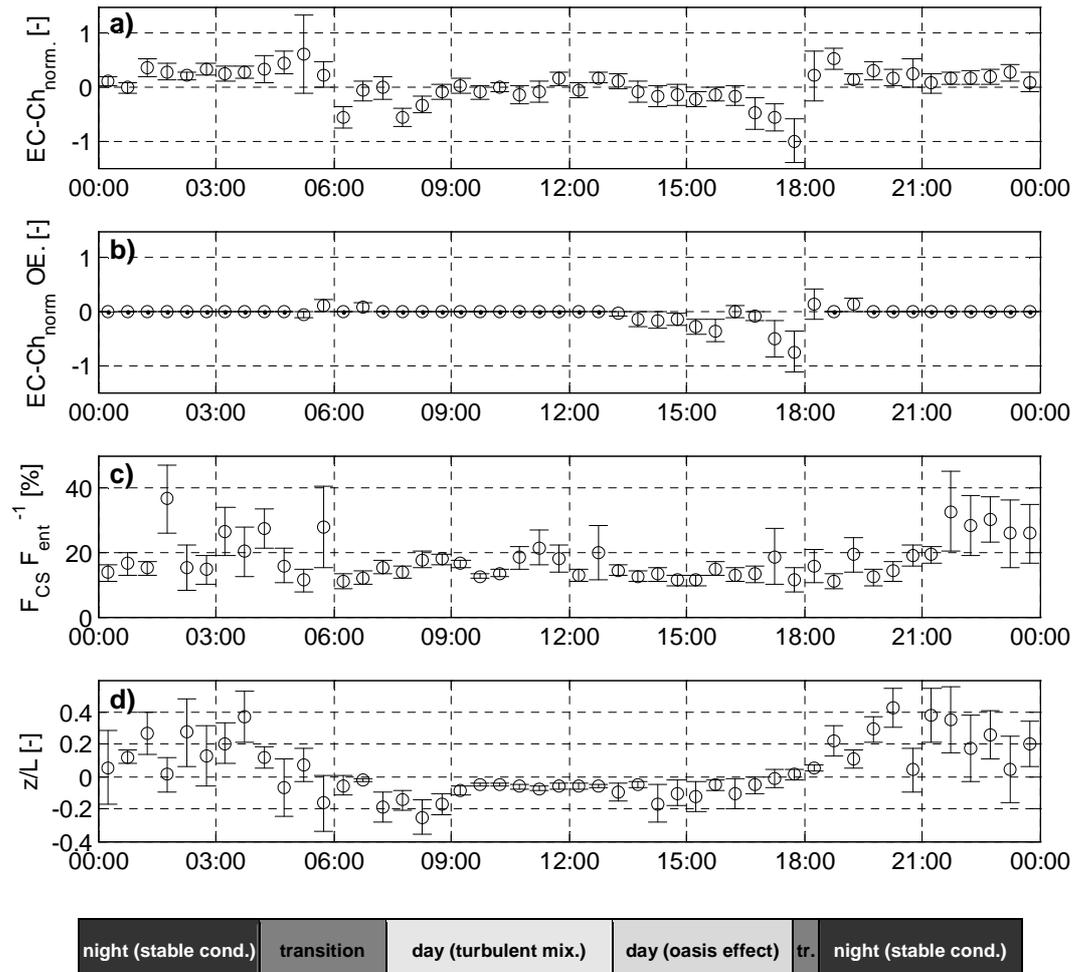


Figure 2. Mean diurnal cycles of a) normalized EC–chamber CO₂ flux differences, b) normalized EC–chamber CO₂ flux differences during times with oasis effect (OE), c) absolute proportion of fluxes by coherent structures and d) the stratification defined by the stability parameter z/L (z : height, L : Obukhov length); the bars below indicate different regimes of atmospheric mixing during the day; incoming shortwave radiation reaches 80 Wm^{-2} at 5:30 and finally at 19:00; time in CET=UTC+1; error bars indicate variation within the 10–day period.

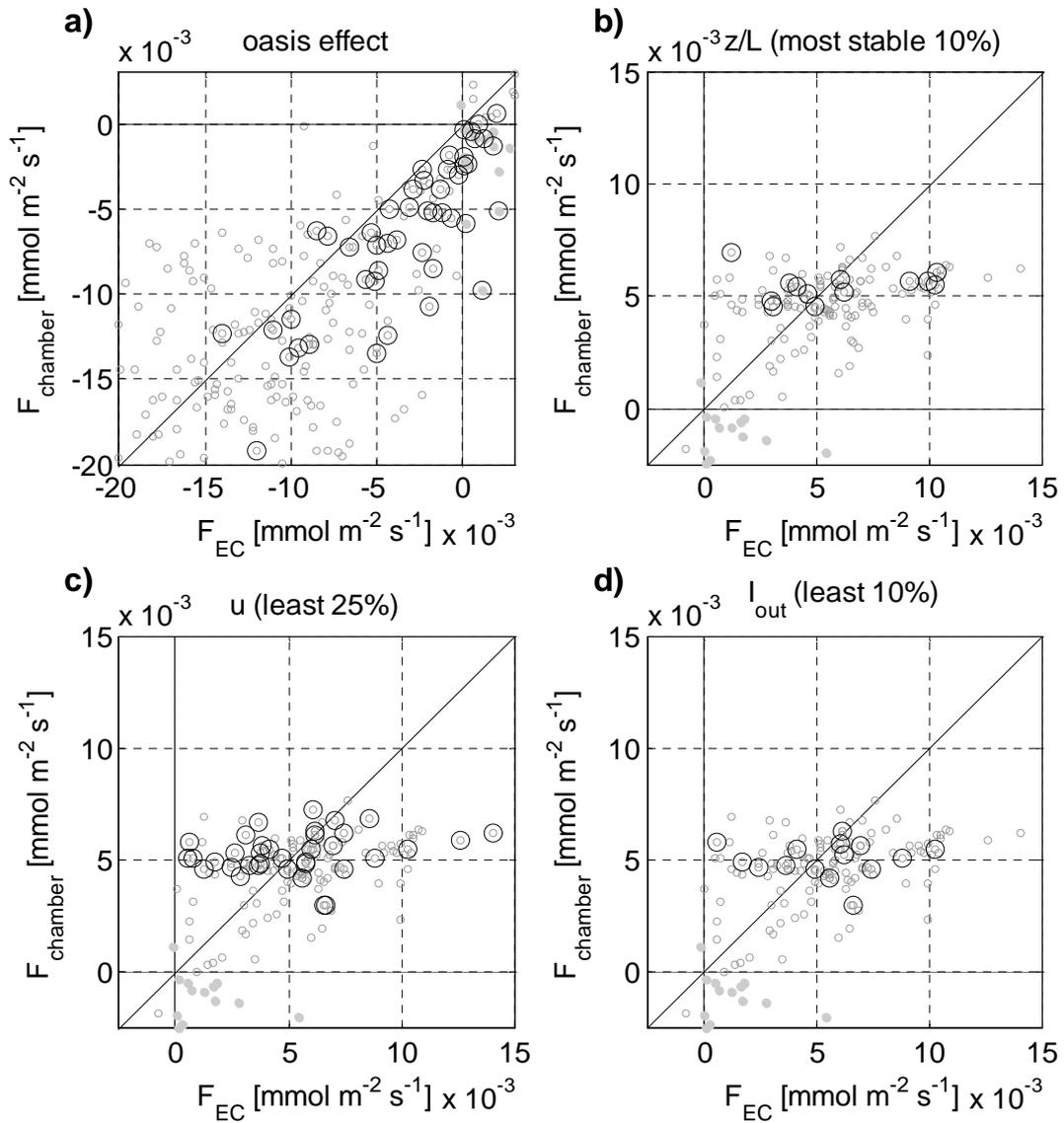


Figure 3. Scatter plot sections of EC- and chamber-determined NEE under particular micrometeorological conditions: a) oasis effect; b) atmospheric stability $z/L > 0.7$; c) wind velocity $u < 0.9 \text{ m s}^{-1}$; d) outgoing long wave radiation $I_{\text{out}} < 319 \text{ W m}^{-2}$ – labeled with large black circles in each case; light grey circles represent fluxes with different directions.

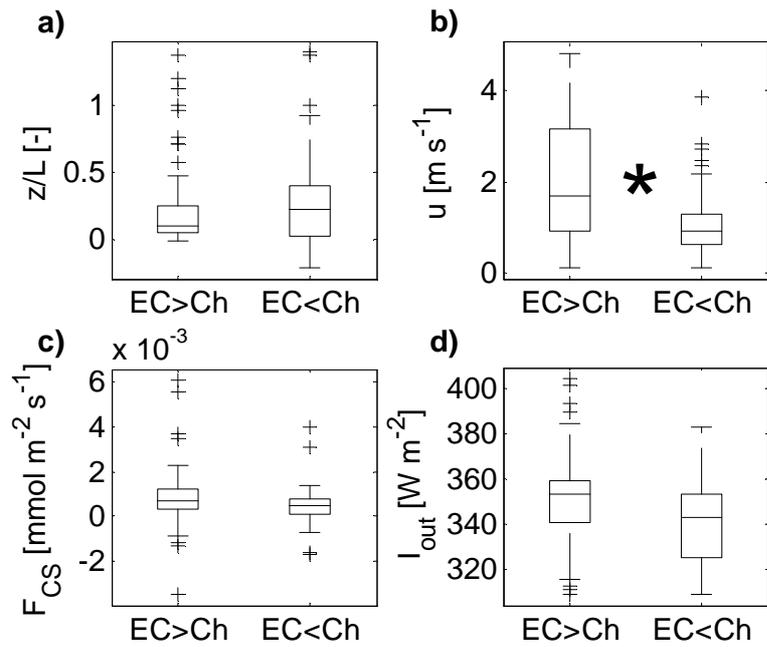


Figure 4. Comparison of a) nighttime atmospheric stability (z/L), b) wind velocity (u), c) CO₂ flux by coherent structures (F_{CS}) and d) long wave outgoing radiation (I_{out}) while either EC or chamber CO₂ fluxes are larger, highly significant difference (Student's t-test for dependent samples, * = $p < 0.01$) found only in case of u (as well as u^*).

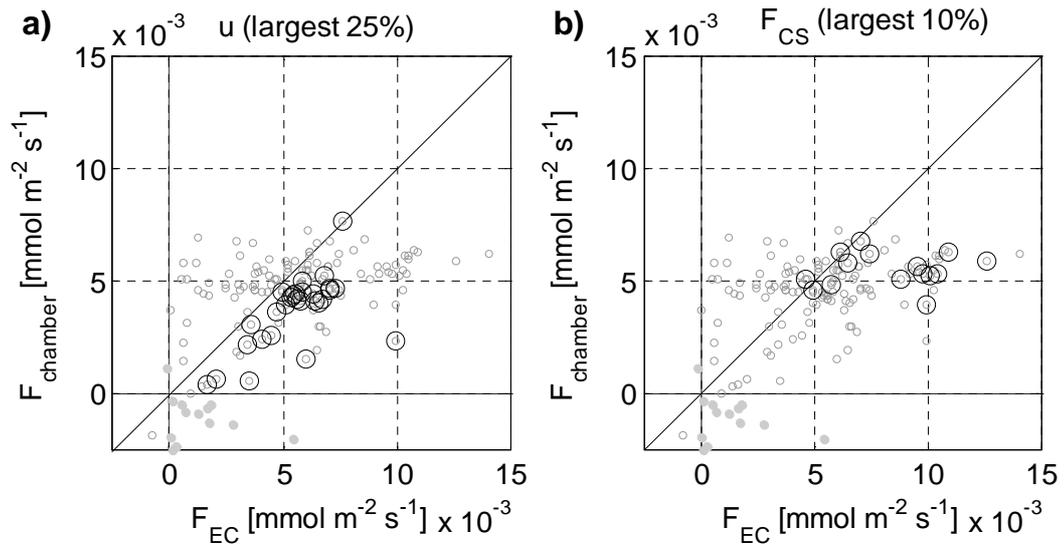


Figure 5. Scatter plot sections of EC– and chamber–determined NEE under particular micrometeorological conditions: a) largest 25 % of the wind velocities ($u > 2.9 \text{ ms}^{-1}$); b) largest 10 % of the fluxes due to coherent structures ($F_{\text{CS}} > 0.0015 \text{ mmol m}^{-2} \text{ s}^{-1}$) – labeled with large black circles in each case, light grey circles represent fluxes with different directions.

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D. INDIVIDUAL CONTRIBUTIONS TO THE JOINT PUBLICATIONS

Other authors contributed to the three manuscripts this cumulative thesis as well. Therefore my own contributions and those of the co-authors to the individual manuscripts are specified in this section.

Appendix A

Riederer, M., Pausch, J., Kuzyakov, Y. and Foken, T., Partitioning NEE for absolute C input into various ecosystem pools by combining results from eddy-covariance, atmospheric flux partitioning and $^{13}\text{CO}_2$ pulse labeling, *Plant Soil*, 390, 61–76, doi:10.1007/s11104-014-2371-7, 2015.

Riederer, M.: 80% (experimental design, accomplishment of experiment, laboratory analyses, data evaluation, manuscript preparation)

Pausch, J.: 10% (experiment support, discussion about experimental design, data analysis and results, comments to improve the manuscript)

Kuzyakov, Y.: 5% (discussion about data analysis and results, comments to improve the manuscript)

Foken, T.: 5% (discussion about results, comments to improve the manuscript)

Appendix B

Riederer, M., Hübner, J., Ruppert, J., Brand, W.A. and Foken, T., Prerequisites for application of hyperbolic relaxed eddy accumulation on managed grasslands and alternative NEE flux partitioning, *Atmos. Meas. Tech.*, 7, 4237-4250, doi:10.5194/amt-7-4237-2014, 2014.

Riederer, M.: 70% (experimental design, accomplishment of experiments, simulation analyses, data evaluation, manuscript preparation)

Hübner, J.: 15% (REA system check, discussion about REA device, simulation and experiment support)

- Ruppert, J.: 5% (discussion about REA device, comments to improve the manuscript; the construction of the REA device is not considered here)
- Brand, W.A.: 5% (air sample isotope analysis)
- Foken, T.: 5% (discussion about data analysis and results, comments to improve the manuscript)

Appendix C

Riederer, M., Serafimovich, A., and Foken, T.: Net ecosystem CO₂ exchange measurements by the closed chamber method and the eddy covariance technique and their dependence on atmospheric conditions, *Atmos. Meas. Tech.*, 7, 1057–1064, doi:10.5194/amt-7-1057-2014, 2014.

- Riederer, M.: 85% (experimental design, accomplishment of experiments, simulation analyses, data evaluation, manuscript preparation)
- Serafimovich, A.: 5% (analysis of coherent structures, comments to improve the manuscript)
- Foken, T.: 10% (discussion about data analysis and results, comments to improve the manuscript)

ERKLÄRUNGEN

Hiermit erkläre ich, dass keine Tatsachen vorliegen, die mich nach den gesetzlichen Bestimmungen über die Führung akademischer Grade zur Führung eines Doktorgrades unwürdig erscheinen lassen.

Hiermit erkläre ich mich damit einverstanden, dass die elektronische Fassung meiner Dissertation unter Wahrung meiner Urheberrechte und des Datenschutzes einer gesonderten Überprüfung hinsichtlich der eigenständigen Anfertigung der Dissertation unterzogen werden kann.

Hiermit erkläre ich eidesstattlich, dass ich die Dissertation selbständig verfasst und keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt habe.

Ich habe die Dissertation nicht bereits zur Erlangung eines akademischen Grades anderweitig eingereicht und habe auch nicht bereits diese oder eine gleichartige Doktorprüfung endgültig nicht bestanden.

Hiermit erkläre ich, dass ich keine Hilfe von gewerbliche Promotionsberatern bzw. -vermittlern in Anspruch genommen habe und auch künftig nicht nehmen werde.

Bayreuth, den 29.09.2014

Michael Riederer