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Preface

This PhD project was carried out at Department of Environmental Engineering, Technical University of Denmark and was supervised by Kim Pilegaard, Klaus Steenberg Larsen and Andreas Ibrom.
The thesis is organized in two parts: the second part contains the findings of the PhD presented in the three papers listed below. The first part of the thesis presents the most important information from the papers in regards to the methods used, the results gained, and it discusses the findings with regard to previous literature. The papers will be referred to in the text by their paper number written with the Roman numerals **I-III.** Brændholt et al., (**I**) has been published in the journal Biogeosciences. Brændholt et al., (**II**) has been submitted to the journal Agricultural and Forest Meteorology. Brændholt et al., (**III**) is a manuscript in preparation for submission.

I **Brændholt, A.,** Larsen, K.S., Ibrom, A., Pilegaard, K., 2017. Overestimation of closed-chamber soil CO_2 effluxes at low atmospheric turbulence. Biogeosciences. 14, 1603-1616.

II **Brændholt, A.,** Larsen, K.S., Ibrom, A., Pilegaard, K. Partitioning of ecosystem respiration in a beech forest. Submitted to Agricultural and Forest Meteorology.

III **Brændholt, A.,** Ibrom, A., Ambus, P., Larsen, K.S., Pilegaard, K. Automated closed-chamber measurements of $\delta^{13}C$ of ecosystem CO_2 fluxes. Manuscript in preparation.

In this online version of the thesis, paper **I-III** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from DTU Environment, Technical University of Denmark, Miljoevej, Building 113, 2800 Kgs. Lyngby, Denmark, info@env.dtu.dk.

In addition, the following conference and journal contributions, not included in this thesis, were also concluded during this PhD study:

Brændholt, A., Ibrom, A., Ambus, P., Larsen, K.S., Pilegaard, K., 2017. Combining an automated closed chamber system with a quantum cascade laser for high-frequency measurements of $\delta^{13}C$ of ecosystem CO_2 fluxes. Poster. EGU General Assembly 2017.

Brændholt A., Larsen K.S., Ibrom A., Pilegaard K., 2016. High-frequency Observations of the Isotopic Composition of Soil, Stem and Root Respiration in a Danish Beech Forest. Poster. 2nd ICOS Science Conference on greenhouse gases and biogeochemical cycles.

Ibrom A., **Brændholt A.,** Pilegaard K., 2016. Surprisingly low frequency attenuation effects in long tubes when measuring turbulent fluxes at tall towers. Poster. EGU General Assembly 2016.

Brændholt A., Larsen K.S., Ibrom A., Pilegaard K., 2016. Overestimation of soil CO_2 fluxes from closed chamber measurements at low atmospheric turbulence biases the diurnal pattern and the annual soil respiration budget. Oral. EGU General Assembly 2016.

Brændholt A., Larsen K.S., Pilegaard K., Ibrom A., 2015. The consequences of the diurnal variation of soil respiration for soil budgets from up-scaled day-time measurements. Poster. Potsdam GHG Flux Workshop 2015.

Ørby P.V., Peel R.G., Skjøth C.A., Schlünssen V., Bønlykke J.H., Ellermann T., **Brændholt A.**, Sigsgaard T., Hertel O., 2015. An assessment of the potential for co-exposure to allergenic pollen and air pollution in Copenhagen, Denmark. Journal paper. Urban Climate. 14, 457-474.

Ørby P.V., Peel R.G., Skjøth C.A., Schlünssen V., Bønløkke J., Ellermann T., **Brændholt A.**, Sigsgaard T., Hertel O., 2014. Assessment of co-exposure to allergenic pollen and air pollution in Copenhagen. Poster. ERS International Congress 2014.

Acknowledgements

This section will be short and to the point.

I would like to thank my supervisors Kim Pilegaard, Andreas Ibrom and Klaus Steenberg Larsen. They are both brilliant scientists and comfortable to be around.

I would also like to thank Poul Sørensen for help with fieldwork during the first part of my PhD and to Jens Schaarup Sørensen, Erik Rønn Lange and Flemming Møller for help with, and company during fieldwork in the last part of my PhD.

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Summary

Ecosystem respiration (R_{eco}) is the second largest flux of CO_2 between the biosphere and the atmosphere. It consists of several components, such as plant respiration and soil respiration (R_{soil}), each of which may respond differently to abiotic factors, and thus to global climate change. R_{soil}, which is the largest component of R_{eco}, is often quantified by the closed-chamber method, where automated chambers can provide information on R_{soil} on a high temporal scale. Although it is a widely used method, some methodological biases are still not fully understood. One emergent issue is the overestimation of closed-chamber fluxes at low atmospheric turbulence. Thus, this potential bias needs to be quantified, and methods need to be developed, to yield correct estimates of R_{soil}. Apart from correct quantification of the flux of CO_2 from R_{soil}, the isotopic composition of C in CO_2 (or $\delta^{13}C$) can reveal important information on the partitioning of R_{soil} into autotrophic and heterotrophic respiration. Traditionally, measurements of $\delta^{13}C$ have been performed by isotope-ratio mass spectrometry, limiting the applicability to low frequency manual measurements. However, recent advances in laser spectroscopy have allowed for real-time measurements of $\delta^{13}C$, thereby providing new ways to investigate the CO_2 fluxes of natural ecosystems at a high temporal scale.

This PhD thesis had three main aims that were all addressed experimentally in a Danish beech forest: The first main aim was to quantify the effect of overestimation of automated closed-chamber soil CO_2 fluxes due to low atmospheric turbulence, and to develop methods to account for this effect. The second main aim was to quantify the individual components of R_{eco} at an annual, seasonal and diel time scale, and the third main aim was to combine an isotope quantum cascade laser with an automated closed-chamber system to yield high temporal $\delta^{13}C$ of chamber-based ecosystem CO_2 fluxes.

To address the first main aim, we measured R_{soil} hourly for one year by automated closed-chambers. The data showed a clear diel pattern of R_{soil} across all seasons with highest rates during nighttime. However, further analysis showed a clear negative relationship between measured flux rates and atmospheric turbulence measured as friction velocity (u_*) above the canopy, suggesting that the measured R_{soil} was overestimated at low atmospheric turbulence. Filtering out data at low u_* values removed, or even inverted, the observed diel pattern, such that the highest fluxes were now observed during daytime, and also led to a substantial decrease of 21 %, depending on u_*

threshold value, in the estimated annual R_{soil}. By installing fans to produce continuous turbulent mixing of air around the soil chambers, we tested the hypothesis that overestimation of soil CO_2 fluxes during low u_* can be eliminated if proper mixing of air is ensured, and indeed the use of fans removed the overestimation of R_{soil} during low u_*.

To address the second main aim, total R_{eco} was measured by the eddy covariance method and the components of tree stem respiration (R_{stem}), heterotrophic R_{soil} from trenched soil, heterotrophic and autotrophic R_{soil} from intact soil, and coarse root respiration (R_{root}) were measured every two hours by automated closed-chambers for one year. We found that the contribution of R_{stem} to total R_{eco} varied across the year, by only accounting for 6 % of R_{eco} during winter and 16 % during summer. In contrast, R_{soil} showed a fairly similar contribution to R_{eco} during winter, spring and summer of 52, 45 and 49 %, respectively, while the contribution increased to 79 % during autumn. By using the trenching method, we found that autotrophic R_{soil} accounted for 34 % of R_{soil} during summer. Diel R_{stem} and R_{root} showed a clear pattern during summer with the highest respiration seen around 13:00-15:00 CET for R_{stem}, and the highest respiration seen from 9:00-15:00 for R_{root}. In contrast, R_{soil} showed the lowest respiration during daytime with no clear difference in the diel pattern between the intact and trenched soil plots. Finally, we calculated the annual R_{soil} for different transects at the site, and found that annual R_{soil} estimated from a previously used transect at the site was underestimated by 20 %, due to R_{soil} of the transect not being representative for the spatial heterogeneity of R_{soil} at the site.

To address the third main aim, an Aerodyne quantum cascade laser for CO_2 isotopes was combined with a LI-8100A/8150 automatic closed-chamber system to yield the $\delta^{13}C$ of CO_2 during automated chamber measurements. The $\delta^{13}C$ of the respired CO_2 for each chamber measurement was determined by the Keeling plot methodology. We found that the $\delta^{13}C$ measured by the laser was influenced by the water vapour and CO_2 concentration of the sample air. However, we quantified these dependencies, and implemented a correction method to yield precise measurements of $\delta^{13}C$. The corrections increased the average $\delta^{13}C$ determined from the Keeling plots by 2.1 and 3.4 ‰ for the water vapour and the CO_2 concentration dependence corrections, respectively. The system was used during a two month campaign where we measured $\delta^{13}C$ every two hours from intact soil, trenched soil, tree stems and coarse roots. The results revealed an average $\delta^{13}C$ of -29.8, -29.7, -30.2 and -32.6 ‰ for

the intact soil plots, the trenched soil plots, the stem plots and the coarse root plots, respectively.

Taken together, the work presented in this PhD thesis shows that periods with low atmospheric turbulence can provide a significant source of error in R_{soil} rates estimated by the closed-chamber techniques and that erroneous data must be filtered out to obtain unbiased diel patterns, accurate relationships to biotic and abiotic factors, and before estimating R_{soil} fluxes over longer time scales. The work also shows that artificial turbulent air mixing may provide a method to overcome the issue with overestimated fluxes, allowing for measurements even at low atmospheric turbulence. Furthermore, the results show that a quantum cascade laser can successfully be combined with an automated closed-chamber system to yield $\delta^{13}C$ of ecosystem CO_2 fluxes at a high temporal scale, but also that the measured $\delta^{13}C$ is highly influenced by water vapour and CO_2 concentration, why a calibration procedure, as developed in this study, is crucial to yield precise measurements of $\delta^{13}C$.

Dansk sammenfatning

Respiration fra økosystemer (R_{eco}) udgør den andenstørste flux af CO_2 fra biosfæren til atmosfæren. Den består af flere komponenter, inklusiv respiration fra planter og respiration fra jord (R_{soil}), der hver især kan have forskelligt respons på abiotiske faktorer såsom temperatur, og derved på de igangværende klimaændringer. R_{soil}, som udgør den største del af R_{eco}, bliver ofte kvantificeret med den lukkede kammermetode, hvor automatiske kamre kan måle R_{soil} ved en høj frekvens. På trods af at det er en udbredt metode, så er visse metodiske biases endnu ikke fuld ud klarlagt. Det gælder blandt andet overestimeringen af målte kammerfluxe ved lav atmosfærisk turbulens. For at kunne lave korrekte estimater af R_{soil}, må effekten af denne bias derfor kvantificeres. Udover præcise målinger af CO_2-fluxen fra R_{soil}, så kan den isotopiske sammensætning af C i CO_2, ofte udtrykt som $\delta^{13}C$, give information om partitioneringen af R_{soil} i autotrof- og heterotrof R_{soil}. Målinger af $\delta^{13}C$ er traditionelt set blevet udført med isotop ratio massespektrometri. Denne metode har dog for det meste begrænset målinger af $\delta^{13}C$ til manuelle målinger ved lav frekvens. Den nye udvikling i laserspektroskopi inden for de seneste år har dog åbnet for muligheden for realtidsmålinger af $\delta^{13}C$ ved høj frekvens, hvilket har givet forskere en ny mulighed for at undersøge CO_2-fluxe i naturlige økosystemer med en høj tidslig opløsning.

Denne ph.d.-afhandling havde tre hovedformål, der alle blev adresseret eksperimentelt i en dansk bøgeskov: Det første hovedformål var at kvantificere effekten af overestimerede CO_2-fluxe målt med et automatisk lukket kammersystem grundet lav atmosfærisk turbulens, og at teste en metode til at kompensere for denne effekt. Det andet hovedformål var at kvantificere de individuelle komponenter af R_{eco} på en årlig, sæsonal og 24-timers tidsskala. Det tredje hovedformål var at kombinere en isotop quantum cascade laser med et automatisk lukket kammersystem for at muliggøre kammerbaserede målinger af $\delta^{13}C$ i økosystem-CO_2-fluxe.

For at besvare det første hovedformål målte vi R_{soil} hver time gennem et år med automatiske lukkede kamre. Resultaterne viste en klar 24-timers cyklus for R_{soil} for alle sæsoner med højst R_{soil} om natten. En videre analyse viste dog et negativt forhold mellem atmosfærisk turbulens, målt som friktionshastigheden (u_*) over kronelaget, og de målte CO_2-fluxe, hvilket indikerede at den målte R_{soil} var overestimeret ved lav atmosfærisk turbulens. Ved at fjerne fluxe målt ved lav turbulens ændredes den 24-timers cyklus, således at de højeste fluxe nu sås om dagen. Desuden resulterede det i et ca. 21 % lavere

estimat af den samlede årlige R_{soil}. Vi testede hypotesen at overestimeringen af jord CO_2-fluxe ved lav u_* kunne fjernes ved at installere ventilatorer, der sørgede for konstant opblanding af luften omkring kamrene. Resultaterne viste at overestimeringen af de målte fluxe ikke længere fandt sted ved lav u_* når ventilatoren sørgede for opblanding af luften.

For at besvare det andet hovedformål målte vi R_{eco} i et år ved hjælp af eddy kovarians-metoden samt komponenterne træstamme-respiration (R_{stem}), heterotrof R_{soil} fra jord hvor bidraget fra rødder blev fjernet ved den såkaldte trenching-metode, heterotrof- og autotrof R_{soil} fra intakt jord, og rod-respiration (R_{root}) hver anden time med automatiske lukkede kamre. Resultaterne viste at bidraget af R_{stem} til R_{eco} varierede over året ved kun at bidrage med 6 % af R_{eco} om vinteren og 16 % om sommeren. Modsat R_{stem}, så viste R_{soil} et mere jævnt bidrag til R_{eco} om vinteren, foråret og sommeren med respektive bidrag på 52, 45 and 49 %, mens bidraget steg til 79 % om efteråret. Ved brug af trenching-metoden fandt vi at autotrof R_{soil} bidrog med 34 % af den samlede R_{soil} om sommeren. 24-timers cyklerne om sommeren for R_{stem} og R_{root} viste et klart mønster med den højeste respiration kl. 13:00-15:00 for R_{stem} og den højeste respiration kl. 9:00-15:00 for R_{root}. R_{soil}, der havde samme mønster for både den intakte- og trenchede jord, havde derimod den laveste respiration om dagen. Vi slutteligt beregnede den samlede årlige R_{soil} for forskellige transekter i skoven, hvorved vi fandt ud af, at et tidligere estimat for den samlede årlige R_{soil} var underestimeret med 20 %, fordi det transekt der blev brugt ikke repræsenterede den rumlige heterogenitet af R_{soil} i skoven godt nok.

For at besvare det tredje hovedformål kombinerede vi en Aerodyne quantum cascade laser for CO_2 isotoper med et LI-8100A/8150 automatisk kammersystem til a måle $\delta^{13}C$ af CO_2 ved automatiske kammermålinger. $\delta^{13}C$ fra det respirerede CO_2 blev for hver kammermåling bestemt med Keeling plot metoden. Resultaterne viste at det målte $\delta^{13}C$ var afhængig af koncentrationen af vanddamp of CO_2 i luften. Vi kvantificerede derfor disse afhængigheder og implementerede en metode til at korrigere rå-data for dermed at opnå præcise målinger af $\delta^{13}C$. Korrektionerne resulterede i højere gennemsnitlige $\delta^{13}C$-værdier bestemt fra Keeling plottene med henholdsvis 2,1 ‰ for vanddampskorrektionen og 3,4 ‰ for CO_2-koncentrationskorrektionen. I en to-måneders målekampagne brugte vi systemet til hver anden time at måle $\delta^{13}C$ fra intakt jord, trenched jord, træstammer og grovrødder. Resultaterne viste respektive gennemsnitlige $\delta^{13}C$ på -29,8, -29,7, -30,2 og -32,6 ‰ for den intakte jord, trenchede jord, træstammerne og grovrødderne.

Samlet set, så viser resultaterne fra denne ph.d.-afhandling at perioder med lav atmosfærisk turbulens er en væsentlig fejlkilde for estimater af R_{soil} målt med den lukkede kammerteknik, og at det er nødvendigt at fjerne målingerne ved lav turbulens for at få korrekte 24-timers cykler, korrekte sammenhænge mellem biotiske og abiotiske faktorer, samt for at få korrekte årlige estimater af R_{soil}. Resultaterne viser også at kunstig luftopblanding kan være en metode til at fjerne problemet med overestimerede fluxe, hvorved målinger kan udføres selv ved lav turbulens. Slutteligt, så viser resultaterne at en quantum cascade laser kan kombineres med et automatisk kammersystem, hvorved der kan måles $\delta^{13}C$ af økosystem CO_2 fluxe på en høj tidslig skala, men også at det målte $\delta^{13}C$ er afhængig af vanddamps- og CO_2-koncentrationen i luften, hvorfor en kalibreringsprocedure, som den der blev udviklet i dette studium, er nødvendig for at få præcise målinger af $\delta^{13}C$.

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1 Background and aims

1.1 Background

The climate is changing and will continue to change in the future (IPCC, 2013). Ecosystem respiration (R_{eco}) plays a crucial role in climate change because it is the second largest flux of CO_2 between the biosphere and the atmosphere, why small changes in ecosystem carbon cycle will have a huge impact on the content of greenhouse gases in the atmosphere (IPCC, 2013). A thorough understanding of ecosystem greenhouse gas cycling is crucial to be able to precisely predict carbon cycling in the future. This can help to better predict the negative impact of climate change for different emission scenarios, thus helping us to take the necessary mitigation actions. For correct quantification of ecosystem CO_2 fluxes, accurate measurements are required, not only for the entire ecosystem, but also for the individual components of ecosystem respiration, since these may respond differently to abiotic factors, and thus to global change (Schimel et al., 2001).

Soil respiration (R_{soil}), which is the largest component of R_{eco}, is often quantified by the closed-chamber method. Although it is a widely used method, some methodological biases are still not fully understood. One emergent issue is the overestimation of chamber fluxes at low atmospheric turbulence (Görres et al., 2016; Juszczak et al. 2012; Lai et al., 2012; Schneider et al., 2009). Thus, this potential bias needs to be quantified, and methods need to be developed, to yield correct estimates of R_{soil}.

Apart from correct quantification of the flux of CO_2 from R_{soil}, the isotopic composition of C in CO_2 can reveal important information on the partitioning of R_{soil}. Traditionally, measurements of $\delta^{13}C$ have been performed by isotope-ratio mass spectrometry, limiting the applicability to low frequency manual measurements (Formánek and Ambus, 2004; Millard et al., 2010). However, recent advances in laser spectroscopy have allowed for real-time measurements of $\delta^{13}C$ (Bowling et al., 2003; Guimbaud et al., 2016; Nelson et al., 2008; Tuzson et al., 2008; Wahl et al., 2011). Due to the recent emergence of the lasers, their full potential has not been explored, why new ways to use the lasers to gather information of $\delta^{13}C$ of ecosystem CO_2 fluxes needs to be developed.

1.2 Aims of the thesis

The overall aims of this PhD thesis was to improve the knowledge of the effect of low atmospheric turbulence conditions on closed-chambers measurements of soil CO_2 fluxes, to use closed-chambers to partition R_{eco}, and to develop a method to measure $\delta^{13}C$ of ecosystem CO_2 fluxes.

The three specific main aims addressed in the thesis were the following:

- The first main aim was to quantify the effect of overestimation of automated closed-chamber for soil CO_2 fluxes due to low atmospheric turbulence, and to develop a method to account for this effect (Brændholt et al., **I**).

- The second main aim was to quantify the individual components of R_{eco} at an annual, seasonal and diel time scale in a temperate beech forest (Brændholt et al., **II**).

- The third aim was to combine an isotope quantum cascade laser with an automated closed-chamber system to yield high temporal $\delta^{13}C$ of chamber-based ecosystem CO_2 fluxes (Brændholt et al., **III**).

1.3 Structure of the thesis

The thesis is structured in the following way:

Chapter 2 presents the state-of-the-art regarding measurements of ecosystem CO_2 fluxes described in Brændholt et al. **(I, II, III**) with focus on closed-chambers.

Chapter 3 describes the methods used in Brændholt et al. **(I, II, III**).

Chapter 4 presents the most important results and points of discussion from Brændholt et al. **(I, II, III**).

Chapter 5 discusses the perspectives of the results obtained in this PhD.

Chapter 6 presents the conclusions of this PhD thesis.

Chapter 7 contains a list of referenced literature.

Chapter 8 contains the three papers Brændholt et al. **(I, II, III**).

2 Measurements of ecosystem CO_2 fluxes

Quantification of ecosystem CO_2 fluxes is a major research area and multiple methods have been developed including the eddy covariance method and various chamber based methods. Eddy covariance is a useful method to quantify net ecosystem exchange of CO_2 (NEE), which can be partitioned into gross primary productivity (GPP) and R_{eco} e.g. by temperature response functions (e.g. Brændholt et al., **II**; Pilegaard et al., 2001; Wofsy et al., 1993). For forests, R_{eco} can be further partitioned into belowground autotrophic and heterotrophic R_{soil} and aboveground respiration from trees and other plants, mainly from respiration from leaves, branches and stems (Brændholt et al., **II**; Hanson et al., 2000; Högberg et al. 2005; Rodeghiero and Cescatti, 2006.

To yield the individual flux components of R_{eco}, chamber based measurements of respiration from soil, leaves, branches and tree stems, can be used (Brændholt et al., **II**; Rodríguez-Calcerrada et al., 2014; Tang et al., 2008; Zhu et al., 2012). Closed-chamber measurements of R_{soil} have been used in multiple studies. However, measurement biases still exist, that can result is wrong estimates of R_{soil}. The presence of the chamber can alter R_{soil}, e.g. by the permanent insertion of the soil collar into the soil (Görres et al., 2016). However, even if the influence of the chamber on R_{soil} is minimal, biases can potentially lead to over- or underestimation of the apparent CO_2 flux measured by the chamber, compared to the actual CO_2 flux from the soil (Anthony et al. 1995; Venterea, 2010; Brændholt et al., **I**; Davidson et al., 2002; Pumpanen et al. 2004; Rochette and Hutchinson, 2005; Ryan and Law, 2005; Conen and Smith, 2000; Hutchinson and Livingston, 1993; Kutzbach et al., 2007; Matthias et al., 1980; Pedersen et al., 2010). The CO_2 flux can potentially be influenced by the atmospheric conditions (e.g. Brændholt et al., **I,** Conen and Smith, 1998; Kanemasu et al. 1974). One such bias is the effect of low atmospheric turbulence, often measured as friction velocity (u_*). A few studies have shown that soil CO_2 fluxes are overestimated during low atmospheric turbulence, with the largest effect typically seen during nighttime (Görres et al., 2016; Juszczak et al. 2012; Lai et al., 2012; Schneider et al., 2009). The cause for the overestimation has been suggested to be due to the build-up of a stratified layer of CO_2 close to the soil, caused by insufficient air mixing. However, due to the chamber movement at chamber closure, the stratified layer breaks down leading to a flush of CO_2 out of the soil (Görres et al., 2016).

Apart from the precise quantification of the CO_2 fluxes, additional information about ecosystem CO_2 cycling and partitioning can be gained from information on the isotopic composition of C of the CO_2 fluxes, often expressed by the δ notation as $\delta^{13}C$ (Bowling et al., 2008). Whereas precise CO_2 concentration measurements have long been performed by online gas analysers, measurements of $\delta^{13}C$ are strongly limited by technology (Brændholt et al., **III)**. Traditionally, $\delta^{13}C$ is determined in the laboratory by the labour intensive isotope-ratio mass spectrometry method (IRMS). This has been used to yield information of $\delta^{13}C$ of R_{soil} by using manually operated closed-chambers (e.g. Formánek and Ambus, 2004). However, high frequency measurements are not possible with IRMS. Thus, IRMS is not suitable to be combined with automated closed-chamber systems, that otherwise have been used for measurements of ecosystem CO_2 fluxes a high temporal scale, e.g. every hour (e.g. Edwards and Riggs, 2003; Koskinen et al., 2014; Liang et al., 2003; McGinn et al., 1998). In contrast to IRMS, novel closed path laser spectroscopy methods, including quantum cascade lasers, have been developed that can perform online measurements of $\delta^{13}C$ (Bowling et al., 2003; Guimbaud et al., 2016; Nelson et al., 2008; Tuzson et al., 2008; Wahl et al., 2011). The quantum cascade lasers have been used for closed-chambers measurements of $\delta^{13}C$ from R_{soil} (Kammer et al., 2011), for open-chamber measurements of $\delta^{13}C$ of tree branch photosynthesis (Gentsch et al., 2014), and for measurements of $\delta^{13}C$ of ecosystem CO_2 fluxes from eddy covariance (Sturm et al., 2012; Wehr et al., 2013). Using quantum cascade lasers for automated closed-chamber measurements have potential challenges due to two inherent properties of the closed-chamber method: Water vapour is present in the chamber air and both the CO_2 concentration and the $\delta^{13}C$ changes during a measurement (Brændholt et al., **III)**. Precise measurement of both the change in CO_2 concentration and $\delta^{13}C$ is crucial, because $\delta^{13}C$ of the respiration source is determined by the Keeling plot methodology by fitting a linear equation to the $\delta^{13}C$ and the reciprocal CO_2 concentration (Keeling, 1958). Closed path laser spectroscopy has, however, been found to be influenced by water vapour and CO_2 concentration dependence, which can cause measured $\delta^{13}C$ to depend on the actual concentrations of both water vapour and CO_2 of the sample air (Pitt et al., 2016; Wen et al., 2013). Thus, to yield precise $\delta^{13}C$ measurements in the wet air with changing CO_2 concentration during closed-chamber measurements, the influence by water vapour and CO_2 concentration dependence must be determined (Brændholt et al., **III)**.

So far, issues related to the measurement of $\delta^{13}C$ of CO_2 fluxes, and to potential measurement biases of the closed-chamber method have been discussed. However, going from the small piece of ecosystem covered by a chamber to the full ecosystem, can cause additional challenges of up-scaling the plot scale measurements in both time and space. R_{soil} may exhibit both a strong seasonal, daily and diel pattern throughout a year, as well as a high spatial heterogeneity in the ecosystem (e.g. Brændholt et al., **I**, **II**; Knohl et al., 2008; Tang et al., 2005; Webster et al., 2008). To capture the seasonal variation in R_{soil}, manual closed-chamber measurements are often performed at regular intervals, and a sufficient number of measurements are performed throughout the ecosystem to capture the spatial variation of the ecosystem (Brændholt et al., **I**, **II**; Davidson et al., 2002; Savage et al., 2008: Savage and Davidson, 2003: Wu et al., 2013). By using a model based on a empirical temperature relationship of R_{soil}, a continuous time series of R_{soil} can be formed for the ecosystem throughout a year (Brændholt et al., **I**, **II;** Lloyd and Taylor, 1994). Whereas the manual measurements provide a good coverage of the temporal variability on a seasonal and annual scale, as well as covering the spatial scale, they do most often not capture the diel variability of R_{soil} because of human nature that most often limit measurements to be performed during daytime working hours (Brændholt et al., **I**). To capture the diel variation of R_{soil}, automated chamber systems can be deployed for unsupervised high temporal scale, e.g. every hour, measurements. However, whereas manual chambers might be limited in temporal resolution, typically a sufficient number of automated chamber is not available to cover the spatial variability in the ecosystem (Brændholt et al., **I**). Going back to the measurement biases of the closed-chamber method, the potential overestimation of measured soil CO_2 fluxes during low atmospheric turbulence can lead to overestimated R_{soil} on both the annual, seasonal, daily and diel scale, thus leading to false conclusions about the magnitude of R_{soil}.

3 Materials and Methods

3.1 Site description

The experiments presented in Brændholt et al. **(I, II, III**) were all carried out at the Danish ICOS research infrastructure site DK-Sor at 40 m a.s.l. (55°29’13’’ N, 55°38’45’’ E). The site has an annual average temperature of 8.5 °C and an annual average precipitation of 564 mm (Pilegaard et al. 2011), which makes the climate temperate maritime. The dense forest at the site is dominated by an almost 100 year old stand of European beech (*Fagus sylvatica* L.) with smaller stands of conifers (Wu et al. 2013). The understory is poorly developed due to the well-developed canopy with a peak LAI of 5.0. Extensive research has been carried out the site mostly focused on the tower-based eddy-covariance measurements of ecosystem CO_2 fluxes have been going on for almost 21 years.

See Pilegaard et al. (2001) and (2011) for a detailed description of the site.

3.2 Overestimation of closed-chamber soil CO_2 fluxes (Brændholt et al., **I**)

To address the first main aim, we measured soil CO_2 flux automatically during a one year campaign with 8 LI-COR long-term CO_2 flux chambers in a multiplexed setup with a LI-8100A Automated Soil CO_2 Flux System and a LI-8150 Multiplexer (LI-COR Environmental, Lincoln, Nebraska, USA) together with u_* calculated from measurements of wind speed by a sonic anemometer at 43 m height above the soil surface. The campaign lasted for one year, with a one-hour measurement cycle, thereby providing 8 soil CO_2 fluxes per hour. In addition, manual closed-chamber measurements of soil CO_2 flux were performed between 09:00–15:00 CET on 12 plots every two weeks.

Soil temperature and soil moisture content were measured at a depth of 5 cm for both the manual and automated measurements.

To test the hypothesis that overestimation of soil CO_2 fluxes during low u_* can be eliminated if proper mixing of air is ensured, we performed an additional 20 day fan campaign of soil CO_2 flux measurements every two hours with the long-term chambers. During the campaign, each plots both experi-

enced 10 days of artificial air mixing by a table fan facing the chamber, and 10 days with ambient conditions.

Data analysis for this and the other parts of the PhD thesis was done using R (R Core Team, 2014). The CO_2 fluxes were calculated on a time and area basis by applying linear regression to the increase in chamber CO_2 concentration during chamber closure time. The CO_2 fluxes for the one year campaign were paired with u_*, and the u_* values were used to create sub-datasets by a u_* threshold filtering technique, where fluxes measured at u_* values lower than a specific threshold value, had been filtered out and removed from the dataset (Aubinet et al. 2000). Twelve different u_* threshold values were used, ranging from 0.1 to 1.2 m s^{-1}. Thus, 12 different sub-datasets each with a specific u_* threshold value were derived from the one year campaign. For each of the sub-datasets, diel ensemble averages of soil CO_2 flux were calculated for each of the four distinct seasons at the site. The annual soil CO_2 flux was obtained for each sub-dataset as well. For the fan campaign, the diel pattern of soil CO_2 fluxes was calculated for both the periods with and without a fan. The manually measured soil CO_2 fluxes were used to parameterize the empirical model by Lloyd and Taylor (1994) with continuously measured soil temperature as model input, from which we derived a continuous time series of mean daily R_{soil} throughout the entire year and calculated monthly and annual R_{soil}.

3.3 Ecosystem CO_2 flux partitioning (Brændholt et al., **II**)

To address the second main aim, we calculated NEE from the eddy covariance measurements performed at a height of 43 meter during one year, and partitioned NEE into GPP and R_{eco}. This resulted in half-hourly values of NEE, GPP and R_{eco} for the entire year as well as in monthly and annual sums.

We used the LI-8100A/LI-8150 automated chambers applied in Brændholt et al. (**I**) to measure respiration from intact soil, trenched soil, coarse roots and tree stems every two hours. For the R_{soil} measurements, we used the LI-COR long-term chambers, whereas we constructed custom made chambers for the 2 root and the 2 stem plots. For two of the soil plots the contribution of living roots to the total R_{soil} was removed by the trenching method, leaving four intact soil plots.

The two root chambers each contained an intact coarse root with a diameter of around 0.5 cm, and the two stem chambers each contained 177 cm^2 of beech stem surface at height of 1.3 m.

The ten chambers were connected to the LI-8100A/LI-8150 in a multiplexed setup, and the system was set up in a repeated automated two-hour cycle during which a measurement of each of the soil, root and stem chambers was performed.

In addition, R_{soil} was measured manually between 09:00–16:00 CET every 2-3 weeks throughout the year. Measurements were performed on 3 different transects in the footprint area of the eddy covariance measurements called the inside fence transect, the south transect and the west transect, respectively. The inside transect consisted of only 12 soil plots positioned within 15 m of the flux tower, whereas the south transect and the west transect consisted of 27 and 45 plots, respectively, distributed throughout the footprint.

All CO_2 fluxes were calculated on a time and area basis by applying a non-linear regression by Hutchinson and Mosier (1981). Different estimates for the annual CO_2 fluxes were calculated for both the manual and automated measurements. For each of the three manually measured transects, we derived daily, monthly and annual R_{soil} with the empirical model by Lloyd and Taylor (1994) similarly to Brændholt et al. (**I**). For the automated measurements, annual mean CO_2 fluxes were calculated for the four intact soil plots, the two trenched soil plots, the two root plots and the two stem plots, respectively. The diel patterns as well as the daily mean and monthly fluxes were calculated as well. R_{stem}, calculated on a stem surface area, was scaled up to the soil surface area by using data on tree density, tree height and diameter at breast height measured at the site.

3.4 $\delta^{13}C$ of ecosystem CO_2 fluxes (Brændholt et al., **III**)

To address the third main aim, we combined an Aerodyne Single CW-Quantum Cascade Laser Trace Gas Analyzer for CO_2 Isotopes (Aerodyne Research Inc. 45 Manning Road Billerica, MA), henceforth called the QCL, with the LI-COR LI-8100A/8150 system used in Brændholt et al. (**I, II**) (Figure 1).

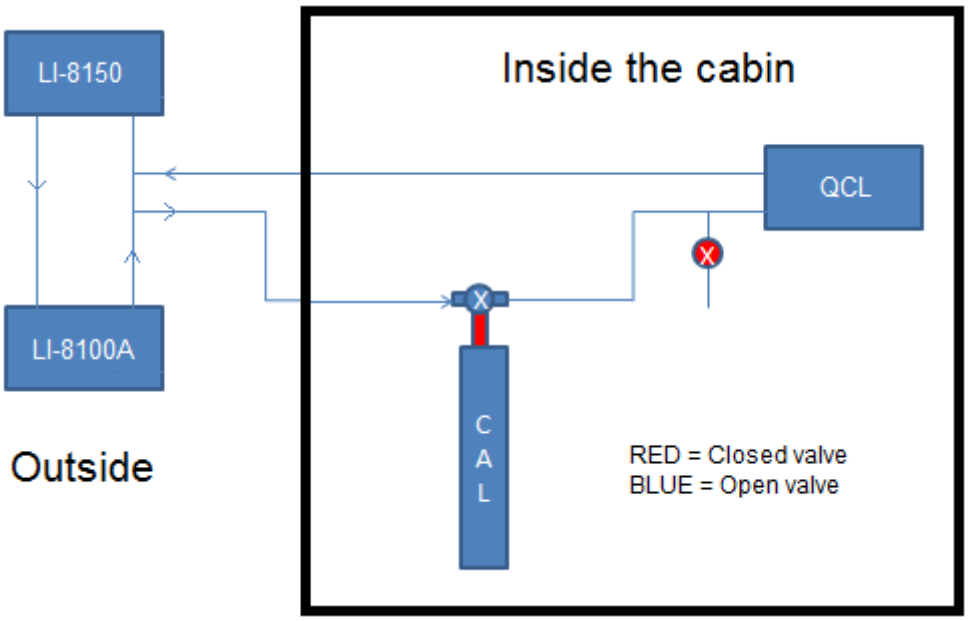

Figure 1. The setup showing the QCL and Li-8100A/8150 combined in parallel. The QCL and calibration gas cylinder (Marked "CAL") were placed inside the air-conditioned cabin at the site and the LI-8100A/8150 was placed outside. The setup is shown as the valves are positioned during a chamber measurement. (Figure and caption adapted from Brændholt et al. (**III**)).

To yield precise $\delta^{13}C$ measurement, we quantified the dependence on $\delta^{13}C$ of water vapour and CO_2 concentration, the so called CO_2 concentration dependence, in the sample air. For water vapour, the effect on the two CO_2 isotopologues $^{16}O^{12}C^{16}O$ and $^{16}O^{13}C^{16}O$, from which $\delta^{13}C$ is calculated, was determined by varying the water vapour content in a gas from a gas cylinder with a known $\delta^{13}C$. The CO_2 concentration dependence was determined by varying the absolute CO_2 concentration, while keeping the $\delta^{13}C$ constant, in a dry gas from a cylinder of known $\delta^{13}C$. The results were used to calculate the vapour pressure broadening correction coefficient, from which we calculated the dry mixing ratios $^{16}O^{12}C^{16}O$ and $^{16}O^{13}C^{16}O$. Following this, the calculated $\delta^{13}C$ was corrected based on the determined CO_2 concentration dependence. Finally, the fully corrected $\delta^{13}C$ was calculated from the calibration performed with a calibration gas of known $\delta^{13}C$ performed every two hours.

The combined system was used during a two month summer campaign where we measured $\delta^{13}C$ and CO_2 fluxes from intact soil, trenched soil, coarse tree roots and tree stems. Four LI-COR long-term chambers were used to measure the CO_2 flux from intact soil, and four chambers were used to measure the CO_2 flux from trenched soil. The custom made chambers also used in Brænd-

holt et al. (**II)** were used to measure root and stem respiration from two plots each.

The $\delta^{13}C$ of the respired CO_2 for each chamber measurement was determined by the Keeling plot methodology. This was done for the different steps of the calibration to allow for examination of the effect of the calibration procedure on the determined $\delta^{13}C$. The CO_2 flux was calculated linearly as well for each measurement.

Using the fully corrected $\delta^{13}C$ values determined by Keeling plots, we calculated the average $\delta^{13}C$ for the entire two months measurement period, as well as the diel pattern of $\delta^{13}C$ for each plot.

4 Results and discussion

4.1 Overestimation of closed-chamber soil CO_2 fluxes (Brændholt et al., **I**)

The results from the one-year campaign showed a diel pattern of u_* across all seasons with highest values during daytime (Fig. 2a, b, c, d).

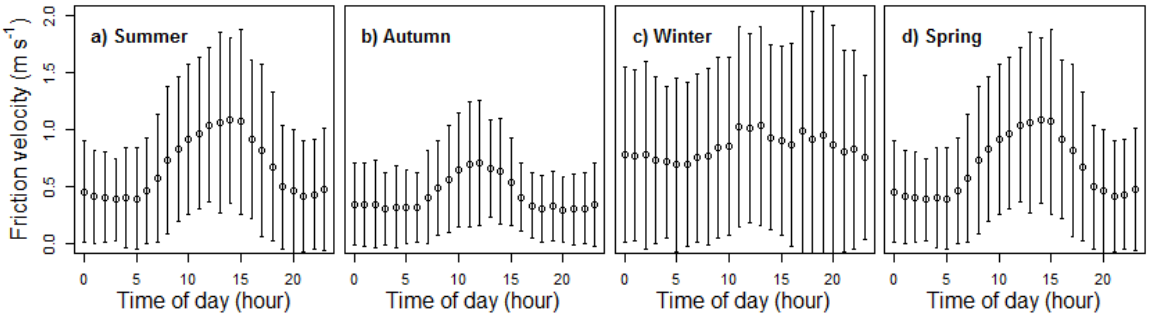

Figure 2. Mean (± standard deviation) diel pattern of friction velocity (u_*) at 43 m above the soil surface for summer (a), autumn (b), winter (c) and spring (d). (Figure and caption adapted from Brændholt et al. (**I**)).

The average diel pattern of soil temperature at 5 cm depth showed a slight diel pattern for summer, autumn and spring with the highest temperatures late in the afternoon or early in the evening (Fig. 3a, b, d), whereas no diel pattern was observed during winter (Fig. 3c).

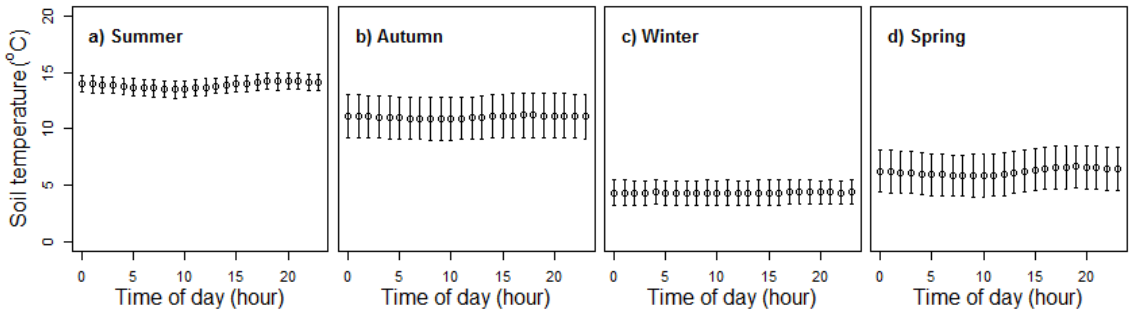

Figure 3. Seasonally averaged diel pattern of soil temperature (± standard deviation) at 5 cm depth for summer (a), autumn (b), winter (c) and spring (d). (Figure and caption adapted from Brændholt et al. (**I**)).

A significant negative relationship was found between u_* and the automated soil CO_2 fluxes for the one year campaign (Fig. 4). Above a u_* threshold value of around 0.7 m s^{-1}, no further decrease in soil CO_2 flux with increasing u_* was observed.

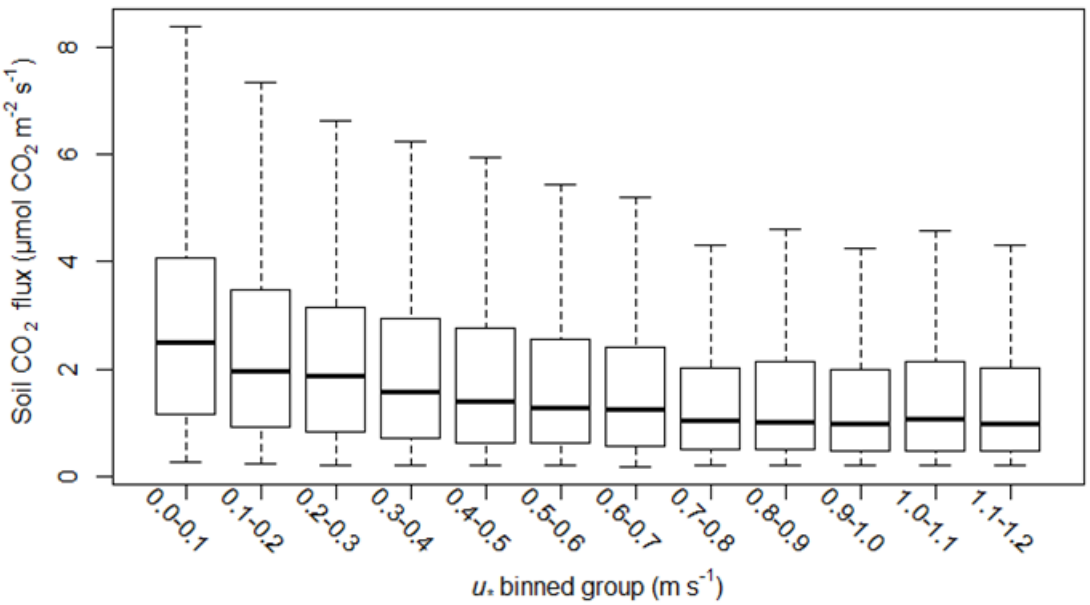

Figure 4. Boxplot of mean hourly soil CO_2 fluxes for the one year campaign plotted against the binned groups of friction velocity (u_*). (Figure and caption adapted from Brændholt et al. (**I**)).

The low atmospheric turbulence typically found during calm nights, both in our and other studies, has been shown to cause a build-up of CO_2 above the soil, because of improper mixing of the layer of air above the surface (Brooks et al., 1997; Stull, 1988). This can result in a lower soil CO_2 flux due to a lower concentration gradient of CO_2 from the soil to the atmosphere (Wohlfahrt et al., 2005; Flechard et al., 2007, Massman et al., 1997). Our chamber measurements, however, showed higher and not lower soil CO_2 fluxes during low u_* (Fig. 4), thereby indicating that the high closed-chamber soil CO_2 fluxes at low u_* is an overestimation, which is in line with a few previous studies (Görres et al., 2016; Koskinen et al., 2014; Lai et al., 2012; Schneider et al., 2009). It has been suggested that the overestimation is due to mixing of the stratified layer of air above the soil surface with air of a lower CO_2 concentration when the chamber moves onto the soil prior to the measurement (Görres et al., 2016), or due to mixing by the internal chamber fan (Lai et al., 2012). This increases the CO_2 concentration gradient between the soil and the atmosphere, which results in the high measured chamber flux (Görres et al., 2016). Thus, closed-chamber CO_2 fluxes measured during low u_* do not represent R_{soil} because steady-state diffusion of CO_2 from the soil to the atmosphere does not take place (Brændholt et al., **I**).

To further investigate the effect of u_* on the CO_2 fluxes, we calculated the mean diel pattern of soil CO_2 flux for each season at different u_* threshold values (Fig. 5). When no u_* filter was applied, the soil CO_2 fluxes showed a clear diel pattern across all seasons with highest fluxes during nighttime where u_* was lowest (Fig. 5a, f, k, p). Applying a successively higher u_* threshold changed the diel pattern, primarily by lowering the overestimated nighttime fluxes, such that the CO_2 flux during summer was only slightly lower in the afternoon. For winter and spring, the diel pattern of soil CO_2 flux changed, such that no apparent difference between nighttime and daytime fluxes was seen (Fig. 5k-5o and 5p-5t, respectively), whereas the highest fluxes during autumn were seen during daytime (Fig. 5f -j). Thus, the overestimation of chamber fluxes due to low u_* works as a selective systematic error that mostly applies to nighttime, which is in agreement with previous studies (Görres et al., 2016; Koskinen et al., 2014; Lai et al., 2012; Schneider et al., 2009). Studies with automated closed-chambers have found a diel hysteresis between soil CO_2 flux and soil temperature, which have been explained as a result of priming that can lead to a peak in R_{soil} decoupled from soil temperature (e.g. Kuzyakov and Gavrichkova, 2010; Phillips et al., 2011; Savage et al., 2013; Tang and Baldocchi, 2005). Our observation of a change in the apparent diel patterns soil CO_2 fluxes due to overestimation of fluxes at low u_* highlights the importance of accounting for this bias, since negligence of the problem may lead to misinterpretation of the relationship between R_{soil} and its physical drivers like temperature and soil humidity, as well as lead to erroneous estimation of lag times between R_{soil} rates and flow of carbon from recent plant assimilates (Brændholt et al., **I)**.

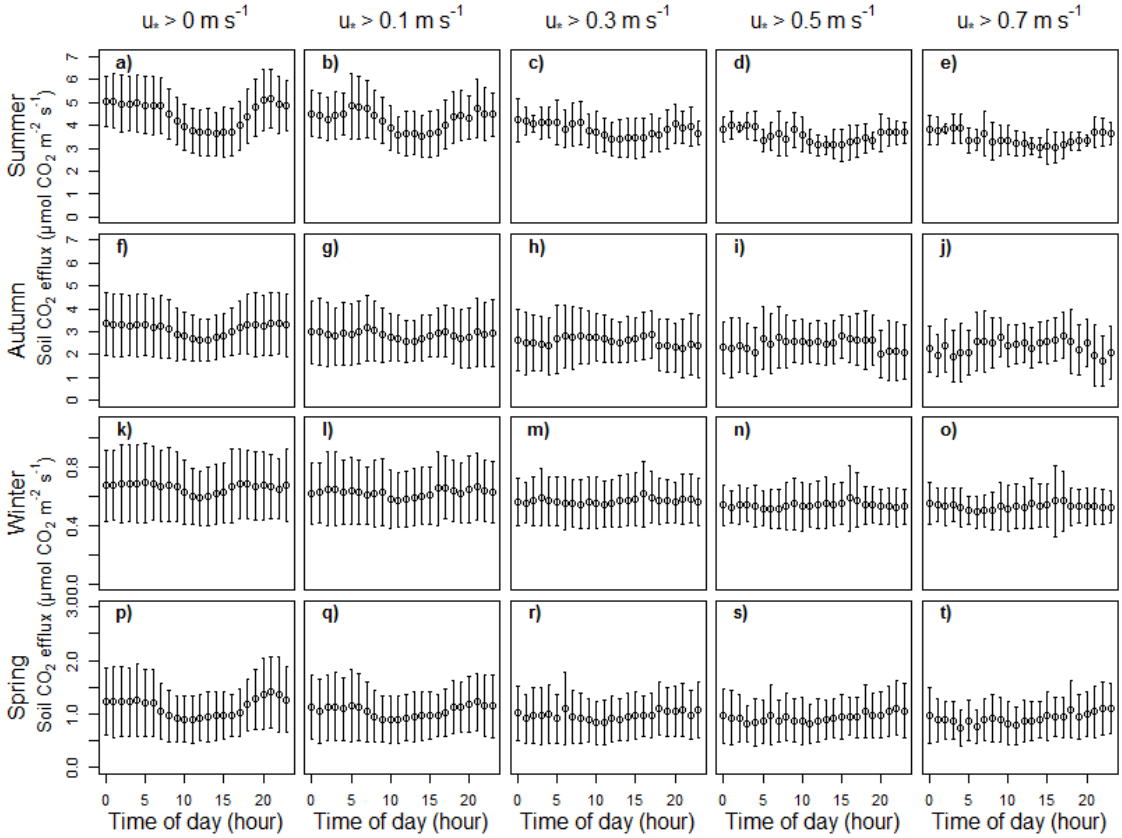

Figure 5. Seasonally averaged diel patterns of automated chamber soil CO_2 fluxes (± standard deviation), at different friction velocity (u_*) threshold values for each of the 4 seasons. From the top, the four rows show the diel patterns for summer, autumn, winter and spring, respectively. From the left, the five collars show the diel patterns for each season at no u_* filtering, a u_* threshold value of 0.1 m s^{-1}, a u_* threshold value of 0.3 m s^{-1}, a u_* threshold value of 0.5 m s^{-1} and a u_* threshold value of 0.7 m s^{-1}, respectively. (Figure and caption adapted from Brændholt et al. (**I**)).

As could be expected from the negative relationship between soil CO_2 flux and u_* (Fig. 4), the estimate of annual soil CO_2 flux decreased in response to increasing the u_* threshold value (Fig. 6). At no u_* filter, the annual soil CO_2 flux estimate was 808.9 g C m^{-2} yr^{-1}. However, at a u_* threshold value of 0.7 m s^{-1}, the annual soil CO_2 flux estimate had decreased by 21 % to 641.7 g C m^{-2} yr^{-1}. Further increasing the u_* threshold value to 1.2 m s^{-1} only decreased the annual soil CO_2 flux estimate by 7 % to 596.9 g C m^{-2} yr^{-1}. The annual CO_2 flux based on the manual chamber measurements was 666.6 g C m^{-2} yr^{-1}. Thus, the automated and manual measurements provide comparable annual CO_2 fluxes, when the overestimation for the automated measurements is accounted for. The manual measurements were performed during daytime (9–15 CET) where u_* was generally high (Fig. 2). Thus, we expect overestimation

of the manual measurements due to low u_* to be minor. This increases the confidence in the much less frequent manual measurements, and shows that measuring during daytime only was not a major source of error for the up-scaling to an annual estimate of soil CO_2 flux (Brændholt et al., **I)**. This is in agreement with Juszczak et al. (2012) who found that when the correct u_* threshold value had been applied, that there was no difference between day-time and nighttime fluxes

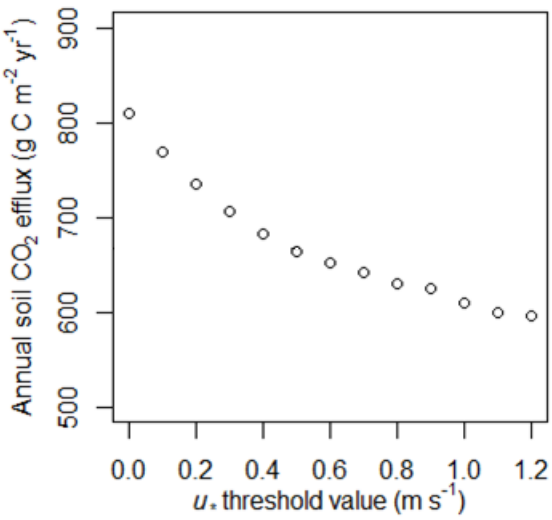

Figure 6. Estimates of annual soil CO_2 flux in response to increasing the friction velocity (u_*) threshold values for the automated chamber measurements during the one year campaign. (Figure and caption adapted from Brændholt et al. (**I**)).

We tested if providing adequate mixing of air around the soil chamber by a fan could remove the closed-chamber bias during low atmospheric turbulence. When no fan was installed, we found a significant negative relationship between soil CO_2 flux and u_*, and the highest fluxes during nighttime, comparable to the one year campaign (Fig. 7a and 7b). However, with fans mixing the air around the soil chambers, the negative relationship changed into a significant positive relationship and changed the apparent diel pattern, such that the highest fluxes now seen during daytime. Using a fan selectively decreased the nighttime (21–03 CET) fluxes the most by 50 %, whereas a decrease of only 26 % was seen for daytime (9–15 CET) fluxes. We argue that the assumption for steady state rate of diffusion of CO_2 out of the soil is closer to being fulfilled with a fan, since the breaking down of the stratified layer of air at low u_* by the chamber in the beginning of a chamber measurement can no longer take place, due to mixing of the air by the fan prior to the

measurement. Thus chamber soil CO_2 measurements are no longer overestimated at low u_*.

A decrease in daytime fluxes was also seen when using a fan. This may indicate that overestimation of fluxes due to low u_* also take place during daytime, which we also observed for the daytime fluxes during the one year campaign (Fig. 5). However, it is possible that the wind induced by the fan introduced a new potential measurement bias, because the rate of diffusion of a gas out of the soil has been found to be sensitive to wind speed at the soil surface, with higher wind speeds leading to an increase in the diffusion rate or even cause advective transport of air out of the soil (Janssens et al., 2000a; Roland et al., 2015). In chambers, where an internal fan creates a higher wind speed in the chamber head space than outside, this effect can cause overestimation of measured soil CO_2 fluxes (e.g. Hanson et al., 1993; Hooper et al., 2002; Le Dantec et al., 1999). The wind speed in the LI-8100A chambers may be low compared to outside conditions, because it does not have internal fan. It is therefore possible that we see the opposite effect of what is seen in chambers with an internal fan, namely that the wind speed in the chamber is lower than outside, resulting in lower measured soil CO_2 flux.

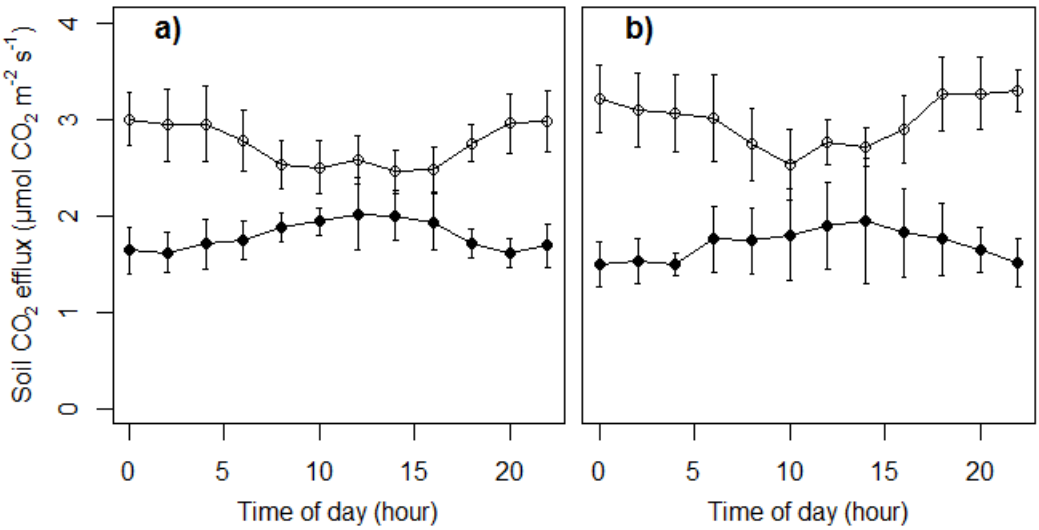

Figure 7. Diel pattern of soil CO_2 flux, measured by the automated chambers during the fan experiment, based on bi-hourly means (± standard deviation). (a) shows the diel pattern for half of the chambers with and without fans, where the first 10 days were with fans (filled circles) and the last 10 days were without fans (open circles). (b) shows the diel pattern with and without fans for the other half of the chambers, where the first 10 days were without fans (open circles) and the last 10 days were with fans (filled circles). (Figure and caption adapted from Brændholt et al. (**I**)).

4.2 Ecosystem CO_2 flux partitioning (Brændholt et al., **II**)

The gap-filled annual NEE calculated from the eddy covariance measurements at a height of 43 m on the flux tower was -391 g C m^{-2} yr^{-1}, and the estimated GPP and R_{eco} was 2272 and 1882 g C m^{-2} yr^{-1}, respectively (Fig. 8). Estimates of annual R_{soil} were calculated from the closed-chamber measurements for each of the three transects (Fig. 8). We found that the annual R_{soil} measured on the inside fence transect was 794 g C m^{-2} yr^{-1}, which was 20 % lower than of the average annual R_{soil} measured on the west and south transects, that had annual R_{soil} of 1024 and 972 g C m^{-2}, respectively. In a previous study at the site, Wu et al. (2013) measured R_{soil} on the inside transect during 5 years and found an average annual R_{soil} of 752 g C m^{-2} yr^{-1}. They argued that R_{soil} had been underestimated, due to the plots having lower R_{soil} than the average R_{soil} of the footprint area. The inside fence transect consisted of 12 plots all positioned at relatively dry and high ground. In contrast, the two newly established west and south transects, consisted of 27 and 45 plots, respectively, spread out evenly in the forest on both higher and lower ground. The annual R_{soil} for the two transects were fairly similar (972 and 1024 g C m^{-2} yr^{-1}), constituting 53 % of R_{eco}. Thus, we argue that the two new transects better represent the spatial variation of R_{soil} in the eddy covariance footprint than the inside fence transect. Knohl et al. (2008) recommended using at least 8 measurement locations spaced randomly throughout the area of interest to get a representative estimate of R_{soil} with sufficient confidence. The two new transects live up to the recommendations by Knohl et al. (2008) by containing 9 and 15 locations, with 3 soil collars each, throughout the eddy covariance footprint, respectively, whereas the inside fence transect does not live up to the recommendations because all the 12 collars are placed at the same location.

We found an average annual R_{stem} for the two automated stem chambers of 258 g C m^{-2} yr^{-1} on a stem surface area basis and 227 g C m^{-2} yr^{-1} on a soil surface area basis (Fig. 8). This accounted for 12 % of R_{eco}, a comparable value to other studies of the contribution of annual R_{stem} to R_{eco} (Ceschia et al., 2002; Janssens et al., 2000b; Tang et al., 2008).

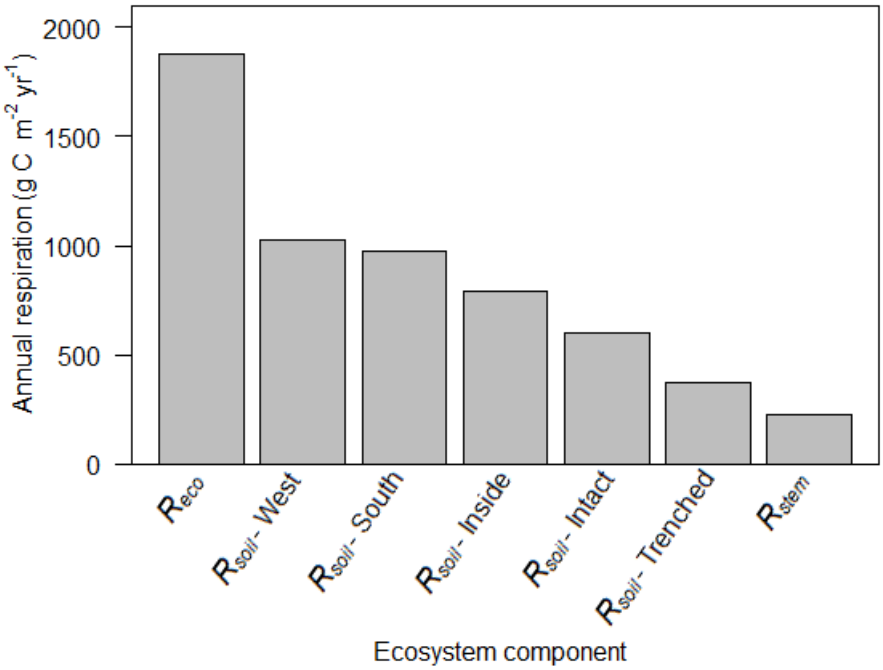

Figure 8. Annual respiration for the different components of the ecosystem. From left to right the bars show: $R_{eco,}$ R_{soil} at the west transect, R_{soil} at the south transect, R_{soil} at the inside fence transect, R_{soil} at the intact soil plots measured by the automated chambers, R_{soil} at the trenched soil plots measured by the automated chambers and R_{stem}. (Figure and caption adapted from Brændholt et al. (**II**)).

R_{eco}, R_{soil} and R_{stem} generally followed the same pattern throughout the year, with highest respiration during the warm summer months (Fig. 9, Fig. 10), which is similar to what is generally observed for temperate forest (Janssens et al., 2000b). However, we found a strong seasonal pattern in the individual component's contribution to R_{eco}, which have been explained by their individual response to temperature and differences in phenology (Migliavacca et al., 2015). R_{stem} experienced a high seasonality in the contribution to R_{eco}, with 6 % during winter and 16 % during summer. The deciduous beech trees shed their leaves during autumn and enter a dormant period until spring, where respiration is limited to only the minimum maintenance respiration (Damesin, 2003). However, in the warm summer growing season, growth respiration, which is mainly determined by phenology, can be a significant part of R_{stem}, which can lead to a larger seasonal difference in R_{stem} than expected from a simple temperature dependence (Lavigne and Ryan, 1997). Our observation of the distinct seasonal pattern of R_{stem} fits well with the phenological stages of growth and dormancy, as well as with the seasonal pattern of R_{stem} found in other temperate forests (Acosta et al., 2008; Edwards et al.,

2002; Griffis et al., 2004; Shibistova et al., 2002; Yang et al., 2012, 2014). In contrast to R_{stem}, R_{soil} from the manual chambers showed an almost similar contribution to R_{eco} during winter, spring and summer of 52, 45 and 49 %, respectively. In contrast to the trees, the microorganisms in the soil do not go into dormancy and can continue to respire throughout the year (Beverly and Franklin, 2015). In autumn there was a higher contribution from R_{soil} of 79 %. During autumn, there is a high input of litter to the soil from shedding of the beech leaves. Thus, it is likely that the input of organic matter to the soil have fuelled the heterotrophic R_{soil}, thereby keeping R_{soil} high, even though the R_{eco} has decreased (Han et al., 2015). This can indeed also been found for other temperate forest ecosystems (DeForest et al., 2009; Hibbard et al., 2005).

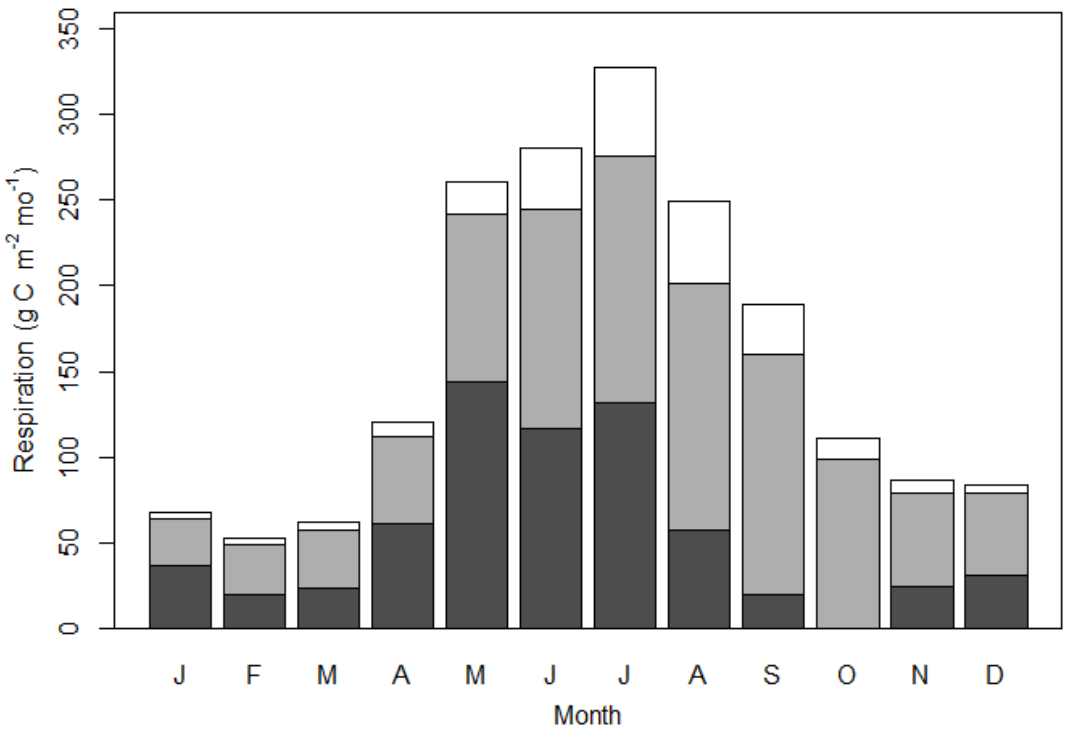

Figure 9. Bar plot showing R_{eco} (the full bar size) and the component respiration of R_{soil} (in grey) and R_{stem} (in white) on a soil surface area basis for each month of 2016. The black bars represent the remaining R_{eco} after R_{soil} and R_{stem} have been subtracted. For the monthly R_{soil}, the average of the manual closed-chamber measurements of the south and west transects is shown. (Figure and caption adapted from Brændholt et al. (**II**)).

The trenching performed for half of the automated chambers allowed for investigating the contribution of heterotrophic and autotrophic R_{soil} across the year. During summer, R_{soil} of the trenched soil plots was only 51 % of R_{soil} for the intact plots. However, during the autumn months this increased to 83 % which was comparable to the pre-trenching level of 77 %. This means that

during summer autotrophic R_{soil} was 49 % of R_{soil}. However, when accounting for the difference in R_{soil} of the plots before the trenching, autotrophic R_{soil} only accounted for 34 % during summer. The variation in the seasonal contribution of autotrophic soil respiration with highest contribution during the plant growing season has been found for several ecosystems, with the main reason being the seasonal pattern of GPP that leads to increased root respiration during the growing season (Beverly and Franklin, 2015; Pumpanen et al., 2015; Hanson et al., 2000). The autotrophic R_{soil} contribution of 34 % during summer is fairly small compared to other forest ecosystems (Brumme, 1995; Epron et al., 2001; Hanson et al. 2000). However, it is possible that decomposing root litter from the severed roots may have contributed to the trenched plots, thereby leading to an overestimated heterotrophic R_{soil}, since the trenching was first performed on 6 April (Díaz-Pinés et al., 2010; Epron et al., 1999; Hanson et al., 2000; Silver et al., 2005; Subke et al., 2006).

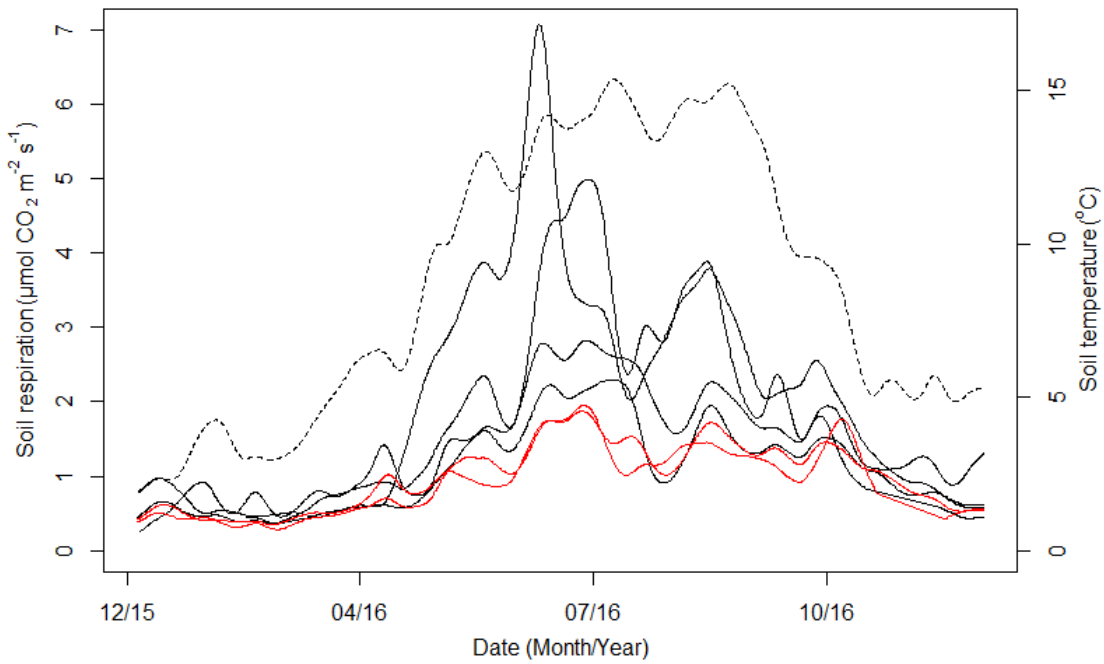

Figure 10. R_{soil} (solid lines) throughout the year measured by the automated closed-chambers and soil temperature at 5 cm depth (dashed line). The black solid lines show R_{soil} for the four plots with intact soil, and the red solid lines show R_{soil} for the two plots with trenched soil. The lines have been smoothed to show a running five day average. (Figure and caption adapted from Brændholt et al. (**II**)).

The automated chamber measurements performed every two hours allowed for investigating the diel patterns of respiration for the various ecosystem components for the different seasons of the year (Fig. 11). The diel pattern of

R_{soil} generally showed the same pattern for both the intact (Fig. 11a, b, c, d) and the trenched soil plots (Fig. 11e, f, g, h). During winter, R_{soil} was generally higher during daytime than during nighttime, whereas no clear diel pattern was observed for spring and autumn. Summer had a diel pattern with low R_{soil} during daytime. Heterotrophic R_{soil} has generally been found to respond to temperature on a diel scale (Chen et al., 2009; Song et al., 2015; Zhang et al., 2015). In contrast, autotrophic R_{soil} has been found to be decoupled from temperature on a diel scale, resulting primarily from priming (Kuzyakov and Gavrichkova, 2010). We, however, saw no difference in the diel pattern of R_{soil} between the intact and trenched soil plots, which could mean that the contributions of autotrophic and heterotrophic R_{soil} do not significantly differ on a diel scale.

Both R_{stem} and R_{root} differed from R_{soil} by showing a clear diel pattern during summer with the highest respiration seen around 13:00-15:00 and 9:00-15:00 CET for R_{stem} and R_{root}, respectively (Fig. 11o, k). This distinct diel pattern of high R_{stem} during the afternoon, also found in other studies, have been explained by a temperature response to the diel pattern of temperature (Acosta et al., 2008; Teskey and Mcguire, 2007). During winter, R_{stem} showed no diel pattern, which might reflect the tree dormancy during this period (Fig. 11m).

The high R_{root} during 9:00-15:00 is consistent with the findings of other studies, where the diel peak have been found to be linked with photosynthesis (Chen et al., 2010; Drake et al., 2008; Lai et al., 2016; Wertin and Teskey, 2008). The peak in R_{root} is consistent with the daily peak in photosynthesis at the site, indicating a link between R_{root} and photosynthesis (Pilegaard et al., 2001). However, the increase in R_{root} have been found to lag after photosynthesis from a few hours up to 4-5 days due to the time it takes to transport the photosyntates from the leaves to the roots (Drake et al., 2008; Kuzyakov and Gavrichkova, 2010; Lai et al., 2016; Tang et al., 2005). Thus, it is possible that R_{root} in our study lags behind photosynthesis with exactly 1 or more days. That no diel pattern was seen during winter and autumn, where no or little photosynthesis occur, could also imply that photosynthesis, at least in part, can alter the diel pattern of R_{root} (Fig. 5i, l).

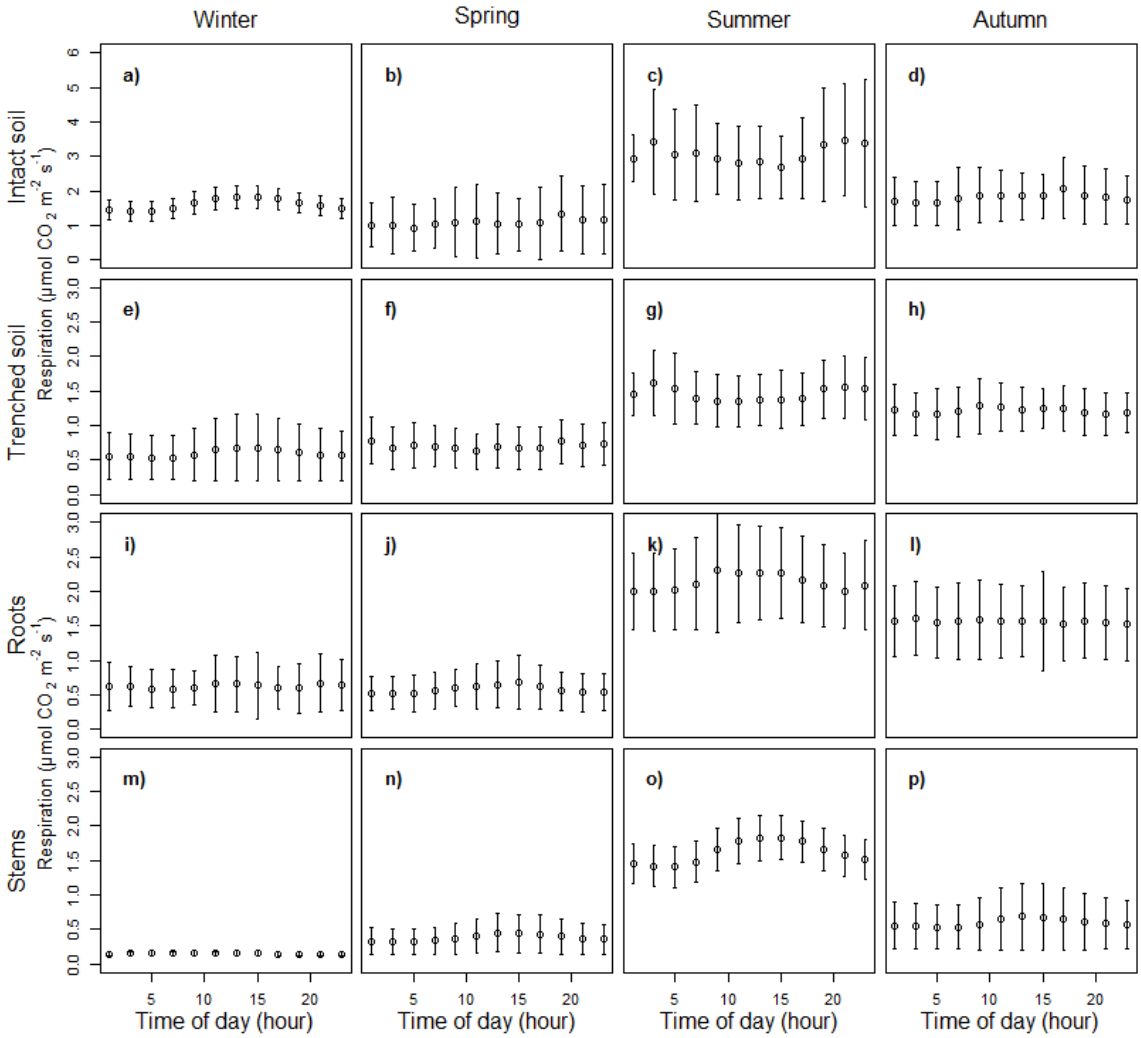

Figure 11. Seasonally averaged diel patterns of R_{soil} for the intact and trenched soil, R_{root} and R_{stem} measured by the automated closed-chambers. Error bars show standard deviation. The seasons of winter, spring, summer and autumn are shown in the four columns from left to right, respectively. The four rows from top to bottom show R_{soil} from intact soil, R_{soil} from trenched soil, R_{root} and R_{stem}, respectively. R_{soil} is shown on a soil surface area basis, while R_{root} and R_{stem} are shown on root surface area and stem surface area basis, respectively. (Figure and caption adapted from Brændholt et al. (**II**)).

4.3 $\delta^{13}C$ of ecosystem CO_2 fluxes (Brændholt et al., **III**)

The water vapour experiment revealed that that the measured mixing ratios of $^{16}O^{12}C^{16}O$ and $^{16}O^{13}C^{16}O$ were lower in wet than in dry air, but also that the two species were not affected equally, which resulted in a negative relationship between measured $\delta^{13}C$ and water vapour content of the sample air (Fig. 12). The results were used to parameterise an equation for both species to calculate the dry mixing rations of a sample with water vapour.

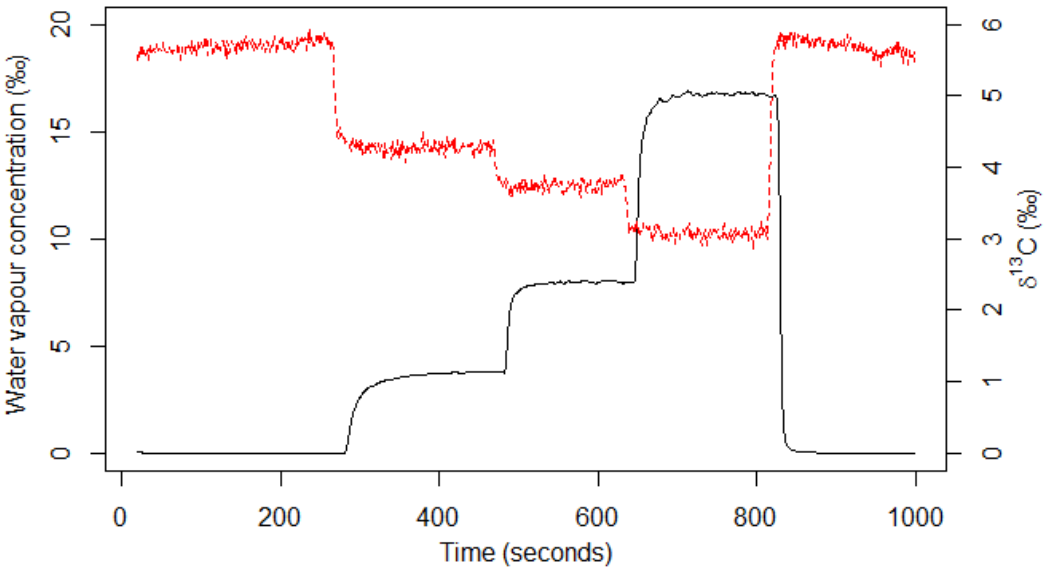

Figure 12: Effect on water vapour (in black) on raw $\delta^{13}C$ (in red) resulting from the effect of water vapour on measured $^{16}O^{12}C^{16}O$ and $^{16}O^{13}C^{16}O$. The figure shows an example of one experiment where the water vapour concentration was varied for the same gas. (Figure and caption adapted from Brændholt et al. (**III**)).

The experiment to test the effect of CO_2 concentration on measured $\delta^{13}C$, when the $\delta^{13}C$ was kept constant, showed through linear regression, a negative relationship between CO_2 concentration and measured $\delta^{13}C$, corresponding to a decrease in $\delta^{13}C$ of 0.54 ‰ per a 100 ppm increase in CO_2 concentration (Fig. 13). From the linear regression we derived an equation to normalise $\delta^{13}C$ to a CO_2 concentration of 400 ppm, thereby removing the effect of CO_2 concentration dependence. The reason for the CO_2 concentration dependence is not yet resolved (McManus et al., 2015). Other studies of CO_2 concentration dependence for closed path laser spectroscopy methods, however, also show varying dependences ranging from no effect found for a ring-down spectroscopy analyser by Vogel et al., (2013) and a quantum cascade laser

developed by Guimbaud et al. (2016), to an increase in $\delta^{13}C$ per a 100 ppm increase of 0.46 and 0.09 ‰, respectively, for two ring-down spectroscopy analysers in a comparison study by Pang et al., (2016), which was opposite of the decrease in $\delta^{13}C$ found in our study.

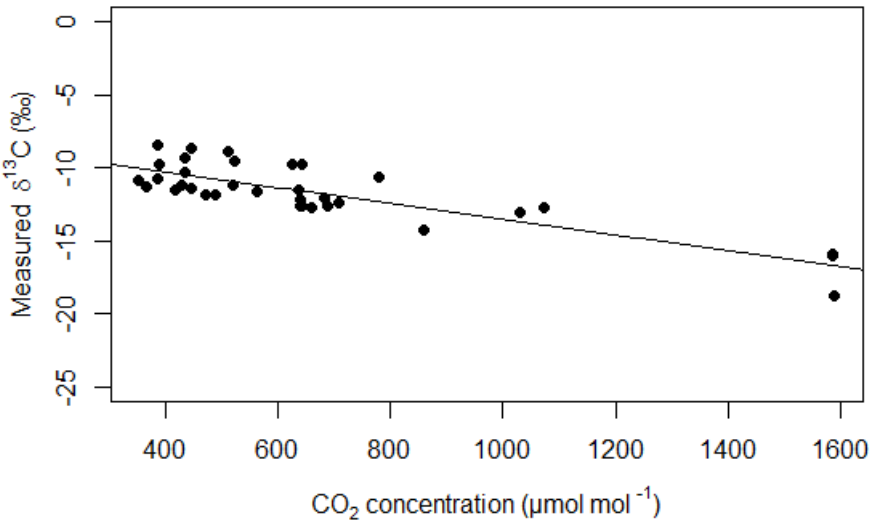

Figure 13: Effect of CO_2 concentration of the sample on measured $\delta^{13}C$. The line shows the fitted linear regression from which we derived an equation used to normalise $\delta^{13}C$ to a CO_2 concentration of 400 ppm. (Figure and caption adapted from Brændholt et al. (**III**)).

Both corrections changed the Keeling plots by increasing the intercept with the y-axis, and thus the determined $\delta^{13}C$ values during the automated closed-chamber campaign. The water vapour correction increased the mean $\delta^{13}C$ for all measurements by 2.1 ‰, and the CO_2 concentration dependence correction further increased the $\delta^{13}C$ by 3.4 ‰. Even though the CO_2 concentration dependence correction only changed the $\delta^{13}C$ slightly, compared to the water vapour correction, it had the highest impact on the $\delta^{13}C$ determined by the Keeling plots, because it decreased the slope of the plots by 14.9 % (Fig. 14). This highlights the importance of the CO_2 concentration dependence correction, because the change in CO_2 concentration and $\delta^{13}C$ during the closed-chamber measurement is inherent to the Keeling plot methodology.

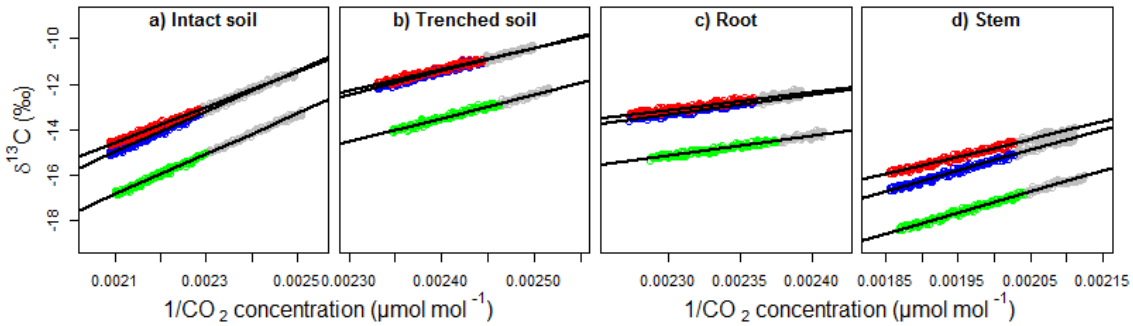

Figure 14. Example of Keeling plots during the automatic chamber campaign for an intact soil measurement (a), a trenched soil measurement (b), a root measurement (c) and a stem measurement (d). For each measurement three data series are shown. The green shows the 300 seconds of raw data for a chamber measurement, where no corrections have been applied to the mixing ratios. The blue shows data where the water vapour correction has been applied, and the red shows data where both the water vapour and the CO_2 concentration corrections have been applied. The grey data points for each data series represents data before the dead band of 120 seconds that were excluded from the Keeling plot. The regression line from the Keeling is included for each data series. All the $\delta^{13}C$ values are calibrated against a standard gas with known $\delta^{13}C$, which on average moved the $\delta^{13}C$ down by 17.85 ‰. (Figure and caption adapted from Brændholt et al. (**III**)).

The two-month campaign yielded 6588 quality checked closed-chamber measurements of $\delta^{13}C$ from intact soil, trenched soil, coarse tree roots and tree stems. We calculated the mean $\delta^{13}C$ for each plot to compare $\delta^{13}C$ of the respired CO_2 for the different ecosystem components (Fig. 15). The average $\delta^{13}C$ for the 4 intact soil plots was -29.8 ± 0.32 ‰, which was similar to the average $\delta^{13}C$ of -29.8 ± 1.2 ‰ for the four trenched soil plots. The lowest $\delta^{13}C$ was seen for the two root plots with an average $\delta^{13}C$ of -32.6 ± 0.78 ‰. $\delta^{13}C$ from heterotrophic respiration of soil organic matter is generally found to be enriched compared to $\delta^{13}C$ from autotrophic respiration, with the resulting $\delta^{13}C$ of R_{soil} from intact soil falling in between, because it is a mix of heterotrophic and autotrophic respiration (Formánek and Ambus, 2004; Millard et al., 2010). Our finding of lower root $\delta^{13}C$ compared to the intact and trenched soil, fits with the depleted $\delta^{13}C$ from roots generally observed (Millard et al., 2010). However, the similar $\delta^{13}C$ for the intact soil, that contain a mix of autotrophic and heterotrophic R_{soil}, and the trenched soil, that ideally only contain heterotrophic R_{soil}, is in disagreement with the higher $\delta^{13}C$ from heterotrophic R_{soil} (Sakata et al., 2007). In the trenched soil the autotrophic contribution was removed by trenching. However, if the trenching was not successful, some roots might still be left in the plot thereby contributing with autotrophic R_{soil}. It is also possible that breakdown of root litter

from severed roots from the trenching, that was only performed two months prior to the campaign, have contributed with CO_2 depleted in $\delta^{13}C$ compared to CO_2 from heterotrophic R_{soil} (Epron et al., 1999; Formánek and Ambus, 2004; Silver et al., 2005; Subke et al., 2006). The average $\delta^{13}C$ value of the two stem plots was -30.2 ± 0.74 ‰, almost similar to the soil plots, but enriched compared to the root plots. Stem CO_2 flux have been found to be composed, not only of respiration, but also of passive diffusion of CO_2 out of the stem (Salomón et al., 2015). This CO_2 can potentially come from R_{soil}, if the CO_2 dissolved in the soil water is being transported by the water up through the stem via the xylem (Bekele et al., 2007; Yavitt et al., 1995). Thus, the measured $\delta^{13}C$ for stem CO_2 flux, which was close to the $\delta^{13}C$ of R_{soil}, could indicate that a large part of the stem CO_2 flux is derived from CO_2 from R_{soil}.

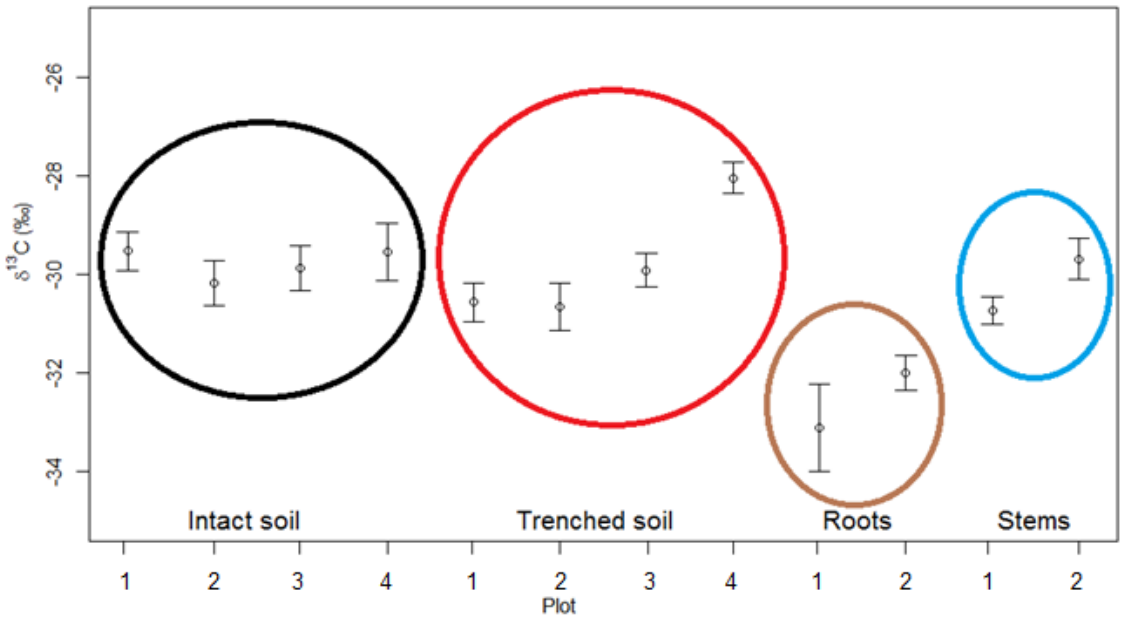

Figure 15. Mean $\delta^{13}C$ the respired CO_2 determined by Keeling plots (± standard deviation) for each of the 12 plots throughout the measurement period. The plots are shown from left to right in the following order: Intact soil 1, 2, 3 and 4. Trenched soil 1, 2, 3 and 4. Root 1 and 2, and stem 1 and 2. (Figure and caption adapted from Brændholt et al. (**III**)).

The average diel patterns of $\delta^{13}C$ for the two months showed a high degree of variability with a clear diel pattern for some plots, when considering the average values, whereas other plots showed no distinct pattern (Fig. 16). $\delta^{13}C$ for the two stems showed no distinct difference between daytime and nighttime (Fig. 16k, l), which is in contract to earlier findings that found a distinct diel pattern with highest $\delta^{13}C$ during daytime (Maunoury et al., 2007). The CO_2 fluxes, however, were highest during daytime, which might

be related to higher temperature (Teskey and Mcguire, 2007). Intact soil 1, 2 and 4 showed a diel pattern with highest $\delta^{13}C$ values during nighttime. A higher contribution of root respiration to total R_{soil} during daytime could explain this. A diel pattern with highest root CO_2 fluxes was indeed seen for root 1 (Fig. 16i), but not for root 2 (Fig. 16j). However, the higher root respiration during daytime is not reflected in a higher CO_2 flux during daytime for the intact soil plots (Fig. 16a, b, c, d). Instead the highest fluxes were seen during nighttime. This might, however, be related to the overestimation of soil CO_2 fluxes measurements during nighttime as found in Brændholt et al. (**I**), which can complicate interpretation of the diel pattern of soil CO_2 fluxes.

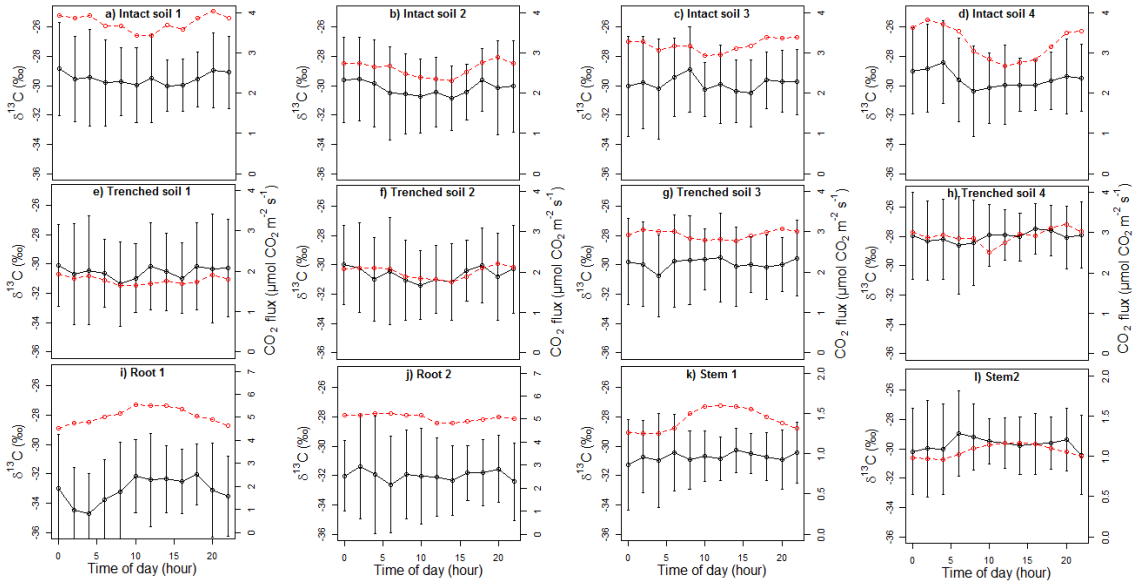

Figure 16: Diel patterns of $\delta^{13}C$ of the respired CO_2 determined by keeling plots (± standard deviation, black points and lines) and CO_2 fluxes (red circles and lines), based on bi-hourly means throughout the measurement period for each of the 12 plots. (a) to (d) show intact soil 1 to intact soil 4, (e) to (h) show trenched soil 1 to trenched soil 4, (i) and (j) show root 1 and root 2, respectively, and (k) and (l) show stem 1 and stem 2, respectively. (Figure and caption adapted from Brændholt et al., (**III**)).

5 Perspectives

The results of this PhD thesis raise new questions and open up new perspectives to future studies. One topic is how to get unbiased measurements of soil CO_2 fluxes during low atmospheric turbulence. Another is the potential future use of laser spectroscopy to measure $\delta^{13}C$ of ecosystem CO_2 fluxes and how the measurements can be improved. Both topics will be discussed in the following.

In Brændholt et al. (**I**), we used a u_* filtering procedure, which we showed could be used to account for the effect of low u_* and we used this procedure in Brændholt et al. (**II**) for closed-chamber measurement of intact and trenched soil. However, the diel patterns for intact and trenched soil were identical (Fig. 11). Thus, the high R_{root} found during daytime was not reflected in the intact soil plots. It is possible that the diel pattern of R_{root} is not pronounced enough to result in a higher daytime R_{soil} for the intact soil. However, it is also possible that overestimation still has an effect on the measured soil CO_2 fluxes that may be the dominant cause of the diel pattern, if the diel pattern of the true R_{soil} is small. The fan experiment, on the other hand (Fig. 7), showed the highest soil CO_2 fluxes during daytime for the intact soil used in Brændholt et al. (**I**), which corresponds with the highest daytime value of R_{root}. However, the artificial mixing of air with a fan is a new method, and it may introduce a new bias as discussed in section 4.1.

One challenge with the u_* filtering procedure is to choose the correct u_* threshold value (Brændholt et al., **I**). In Brændholt et al. (**II**) we subjectively chose the u_* threshold value by visual inspection of a scatter plot of CO_2 fluxes versus u_*, as where the negative relationship between flux and u_* levelled off. A similar method has also been used for determination of the u_* threshold value for eddy covariance measurements. However, several objective statistical methods have also been developed, which could be interesting to test in future studies of closed-chamber soil CO_2 fluxes (Gu et al., 2005). Although we showed that a u_* filtering procedure is a useful method, it consequently leads to data gaps, and thus loss of data. Methods therefore need to be developed to allow for unbiased chamber measurements also during periods with low atmospheric turbulence (Brændholt et al., **I**). Accurate CO_2 fluxes during low u_* are of special importance since the eddy covariance method does not work during these conditions.

The results from the fan experiment in Brændholt et al. (**I**) showed promising results in terms of removing the negative relationship between u_* and soil CO_2 fluxes and we believe that this method have a future potential.

It is possible that the design of the closed-chamber may also influence the degree of overestimation of CO_2 fluxes. Görres et al. (2016) argued that unbiased fluxes during low atmospheric turbulence might be possible if certain design criteria are met that ensure that the stable atmospheric layer of air above the soil surface is not broken up during a measurement. These criteria include a low chamber height of less than 20 cm and a slow chamber closing speed in the horizontal plane, both aiming at keeping the chamber in the same horizontal plane, where there is no steep CO_2 gradient. 5 of the LI-COR long-term CO_2 flux chambers used in Brændholt et al. (**I**) were the 8100-104 model and 3 were the 8100-101 model. The 8100-104 model closes in the horizontal plane, whereas the 8100-101 model closes onto the soil collar from a higher position above the soil surface. However, we observed no discernible difference in the overestimation of the measured fluxes by the two chamber types. A common design feature of the two chamber types is that the air inlet and outlet are placed at the top of the dome shaped chamber. This means that during pre-purge, when the chamber moves on to the soil collar, air from a different place than the soil surface in the soil collar, is pumped to and from the chamber. This could potentially lead to mixing of the air at the soil surface in the collar with air further away from the collar. We have initiated a new experiment to test if a different position of the chamber inlet could potentially reduce or eliminate the effect of the flux overestimation during low turbulence. Instead of having the chamber inlet in the chamber itself, we have moved the inlet to the soil collar, to ensure that only air directly at the soil collars is suck into the system. As of today the, the experiment has started. However, we still await the first results.

So far we have only considered closed-chambers for measurement of R_{soil}. However, dynamic open-chambers, also called flow-through chambers, where the flux is calculated based on the difference in the amount of CO_2 of the inlet and outlet air at steady-state could also be considered. These chambers provide an alternative method to measure soil CO_2 fluxes, although biases also exist for the open soil chambers (Davidson et al., 2002). Some of these chambers are permanently placed on the soil, where they provide continuous soil CO_2 flux measurements. If lateral diffusion is negligible, then all the CO_2 produced from R_{soil} must in theory diffuse into the chamber, except from leaching of dissolved organic and inorganic carbon in some ecosystems (Kin-

dler et al. 2011). This could potentially eliminate the flux overestimation when using closed-chambers caused by the disruption of the stratified layer of CO_2 above the soil surface. Thus, for a continuously operated open-chamber system, there might be a better coupling between R_{soil} and measured soil CO_2 flux. We suggest an experiment where closed-chambers and open-chambers are operated in parallel at the same site, to test the potential of open-chamber measurements of soil CO_2 fluxes during low atmospheric turbulence.

We successfully used the QCL to yield $\delta^{13}C$ of ecosystem CO_2 fluxes determined by the Keeling plot methodology (Brændholt et al., **III**). However, issues regarding the QCL were encountered along the way. One issue was an unexpected large drift in the $\delta^{13}C$ that sometimes accounted for 5 ‰ or more between the bi-hourly calibrations with a calibration gas. We accounted for the drift by linear interpolation between two adjacent calibrations, and removed data when the $\delta^{13}C$ was higher than 2.5 ‰ between two adjacent calibrations (Brændholt et al., **III**). However, a variation in the drift between two adjacent calibrations that might deviate from the linear interpolation is possible. This could potentially cause low accuracy of the calibrated $\delta^{13}C$, which in turn would lower the accuracy of the $\delta^{13}C$ determined from the Keeling plots. As seen e.g. for the diel pattern of $\delta^{13}C$ in Fig. 16, the standard deviation in the determined $\delta^{13}C$ values, shown as error bars, is large, which could be caused by the drift. Quantum cascade lasers have been found to be sensitive to temperature (Guillon et al., 2012; Tuzson et al., 2008). We tested to see if a temperature relationship could be found between the $\delta^{13}C$ and temperature, which could potentially be used to correct for temperature in the post processing of $\delta^{13}C$. However, no such relationship was found. For future studies the frequency of calibrations can be increased to better describe the drift, thereby increasing the accuracy of the calibrated $\delta^{13}C$.

6 Conclusions

The focus of this PhD thesis was to improve the knowledge of the effect of low atmospheric turbulence conditions on closed-chambers measurements of soil CO_2 fluxes, to use closed-chambers to partition R_{eco}, and to develop at method to measure $\delta^{13}C$ of ecosystem CO_2 fluxes for the purpose of partitioning.

One year of measurements with automated closed-chambers showed that periods with low atmospheric turbulence can provide a significant source of error by leading to overestimated R_{soil} rates. However, by using a u_* filtering procedure, we showed that reliable estimates of R_{soil} could be obtained to yield unbiased diel patterns and reliable estimates of R_{soil} over longer time scales. An additional campaign also showed that artificial turbulent air mixing may provide a method to overcome the issue with overestimated fluxes, allowing for measurements even at low atmospheric turbulence.

Furthermore, the results of the PhD showed that a quantum cascade laser can successfully be combined with an automated closed-chamber system to yield $\delta^{13}C$ of ecosystem CO_2 fluxes at a high temporal scale, but also that the measured $\delta^{13}C$ is highly influenced by water vapour and CO_2 concentration, why a calibration procedure, as presented in this study, is crucial to yield precise measurements of $\delta^{13}C$.

Based on the findings of this PhD, we recommend that any analysis of soil CO_2 fluxes measured by automated closed-chambers must consider overestimation of fluxes at low atmospheric turbulence, to yield unbiased estimates of R_{soil}. The results also highlight the need for methodological developments, which will allow for unbiased chamber measurements to be made also during low atmospheric turbulence.

Although the quantum cascade laser was installed in a highly temperature-controlled cabin in the field, the instrument still showed a relatively large, unexplained stochastic drift, which caused a relatively high level of noise in the data far away from the theoretical precision of the instrument. This lack of stability may still represent one of the biggest challenges with the application of these novel isotope measurement techniques under field conditions.

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8 Papers

I **Brændholt, A.,** Larsen, K.S., Ibrom, A., Pilegaard, K., 2017. Overestimation of closed-chamber soil CO_2 effluxes at low atmospheric turbulence. Biogeosciences. 14, 1603-1616.

II **Brændholt, A.,** Ibrom, A., Larsen, K.S., Pilegaard, K. Partitioning of ecosystem respiration in a beech forest. Submitted to Agricultural and Forest Meteorology.

III **Brændholt, A.,** Ibrom, A., Ambus, P., Larsen, K.S., Pilegaard, K. Automated closed-chamber measurements of $\delta^{13}C$ of ecosystem CO_2 fluxes. Manuscript in preparation.

In this online version of the thesis, **paper I-III** are not included but can be obtained from electronic article databases e.g. via www.orbit.dtu.dk or on request from.

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