Overestimation of closed chamber soil CO_2 effluxes at low atmospheric turbulence

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Abstract.

Soil respiration (R_s) is an important component of ecosystem carbon balance and accurate quantification of the diurnal and
10 seasonal variation of R_s is crucial for correct interpretation of the response of R_s to biotic and abiotic factors, as well as for estimating annual soil CO_2 efflux rates.

In this study, we measured R_s hourly for one year by automated closed chambers in a temperate Danish beech forest. The data showed a clear diurnal pattern of R_s across all seasons with higher rates during night-time than during day-time. However, further analysis showed a clear negative relationship between flux rates and friction velocity (u_*) above the
15 canopy, suggesting that R_s was overestimated at low atmospheric turbulence throughout the year due to non-steady state conditions during measurements. Filtering out data at low u_* values removed or even inverted the observed diurnal pattern, such that the highest effluxes were now observed during day-time, and also led to a substantial decrease in the estimated annual soil CO_2 efflux.

By installing fans to produce continuous turbulent mixing of air around the soil chambers, we tested the hypothesis that
20 overestimation of soil CO_2 effluxes during low u_* can be eliminated if proper mixing of air is ensured, and indeed the use of fans removed the overestimation of R_s rates during low u_*. Artificial turbulent air mixing may thus provide a method to overcome the problems of using closed chamber gas exchange measurement techniques during naturally occurring low atmospheric turbulence conditions. Other possible effects from using fans during soil CO_2 efflux measurements are discussed. In conclusion, periods with low atmospheric turbulence may provide a significant source of error in R_s rates
25 estimated by the use of closed chamber techniques and erroneous data must be filtered out to obtain unbiased diurnal patterns, accurate relationships to biotic and abiotic factors, and before estimating R_s fluxes over longer time scales.

1 Introduction

Soil respiration (R_s) in terrestrial ecosystems is the second largest flux of CO_2 after gross photosynthesis and was found to account for 63 % of ecosystem respiration on average in a study of 18 European forest (Janssens et al., 2000; Raich and
30 Schlesinger, 1992). R_s may exhibit both a strong seasonal and diurnal pattern (e.g. Janssens et al., 2000; Tang et al., 2005)

and accurate measurement of R_s at various time scales is thus important to correctly estimate this flux component, i.e. periodic over- or underestimation of R_s may lead to huge errors in annual ecosystem CO_2 budget estimates.

CO_2 produced in the soil must in the long term be emitted from the soil surface (Maier et al., 2011), although in some ecosystems, leaching of dissolved organic and inorganic carbon may occur (Kindler et al. 2011). R_s is therefore often
5   measured as soil CO_2 efflux by the closed chamber method, which relies on Fick's law of diffusion and steady-state diffusion rate conditions (Gao and Yates, 1998). Under these conditions, the flux rate can be calculated from the increase in chamber CO_2 concentration during the chamber deployment period.

To estimate annual R_s on a site, manual chamber measurements are often performed at regular intervals (e.g. twice a month) at multiple plots, which ideally encompass the temporal and spatial variation in R_s in the studied ecosystem. For logistic
10   reasons manual measurements are, however, most often performed during day-time working hours and therefore do not capture the diurnal variation. To capture the diurnal variation, diurnal measurements campaigns at a few plots are often performed at a high temporal resolution (e.g. every 1-2 hour) but the number of campaigns over a full year may often be limited. Using campaign-wise diurnal cycle patterns for the entire year may cause biases, since the diurnal pattern of R_s may itself exert seasonal differences, which may not be captured during a limited number of diurnal measurement campaigns
15   (Ruehr et al., 2010).

The assumption behind the usage of closed chambers for estimating soil CO_2 efflux may also be challenged and a number of potential biases have been identified that may cause an over- or underestimation of the true soil CO_2 efflux (Davidson et al., 2002; Pumpanen et al. 2004; Rochette and Hutchinson, 2005; Ryan and Law, 2005). Firstly, the increase in chamber headspace CO_2 concentration during measurements decreases the concentration gradient between the soil and chamber
20   headspace, thereby theoretically decreasing the apparent soil CO_2 efflux according to Fick's law of diffusion (Gao and Yates, 1998). Consequently it has often been found that applying a linear fit to the increase in chamber CO_2 concentration leads to a systematic underestimation of the measured CO_2 efflux compared to the true efflux (Anthony et al. 1995; Venterea, 2010). To correct for this methodological error a non-linear fit can be applied to the increase in chamber headspace CO_2 concentration to estimate the CO_2 efflux at time zero, where chamber headspace CO_2 concentration is at ambient level.
25   Though theoretically sounder, non-linear fits have been found to increase the uncertainty of the flux estimate (Venterea et al. 2009).

Many chambers are placed on a soil collar permanently installed a few cm into the soil to secure a good sealing of the chamber to the soil surface, thus preventing lateral transport of air into or out from the chamber in the top soil (Healy et al., 1996; Hutchinson and Livingston, 2001; Livingston and Hutchinson, 1995). However, the soil collar may cause a
30   disturbance to the soil e.g. by changing the microenvironment, severing plant roots or even increasing root growth (Görres et al., 2015). These disturbances of the soil can potentially change the R_s inside the collar compared to undisturbed soil.

Placing the chamber on the soil before a measurement may also lead to a disturbance in the diffusion-driven soil CO_2 efflux. This can either lead to a flush of CO_2 from the soil pores into the chamber headspace resulting in a higher soil CO_2 efflux

(Matthias et al., 1980; Hutchinson and Livingston, 1993) or lead to horizontal transport of gas in the soil resulting in a lower soil CO_2 efflux (e.g. Conen and Smith, 2000; Kutzbach et al., 2007; Pedersen et al., 2010).

The conditions of the atmosphere surrounding the chamber may also influence the measured CO_2 efflux. An over- or under-atmospheric pressure in the chamber headspace can act to either suppress or increase the CO_2 efflux respectively (Kanemasu

5   et al. 1974). To maintain ambient pressure in the chamber headspace, closed chambers often have a vent connected to the atmosphere (e.g. Hutchinson and Mosier, 1981; Savage and Davidson, 2003; Xu et al., 2006). High wind speeds moving over the vent tube may lead to a pressure drop in the chamber headspace, due to the Venturi effect, thereby leading to flux overestimation (Conen and Smith, 1998). Correct vent design has, however, been found to eliminate this effect (Xu et al., 2006).

10 While the effects mentioned above are well described, the effect of atmospheric turbulence during closed chamber measurements has received much less attention. Recently, a few studies have demonstrated that low friction velocity (u_*), a measure of atmospheric turbulence, can lead to overestimation of fluxes measured by closed chambers, and that it is especially a problem during night-time where u_* typically is lowest (Görres et al., 2015; Juszczak et al. 2012; Lai et al., 2012; Schneider et al., 2009). It has been suggested that the overestimation of chamber fluxes is because a stratified layer of

15 CO_2 builds up at the soil surface during periods of low u_*, but is broken down by the chamber movement at closure (Görres et al., 2015).

The potential overestimation of soil CO_2 effluxes due to low u_* has become relevant especially in recent years when high-frequency soil CO_2 efflux measurements have become more widespread by the emergence of commercially available automated closed chamber systems, and increasing integration and usage of chamber flux technologies in international

20 research infrastructures, such as ICOS (Integrated Carbon Observation System), which aims to quantify the greenhouse balance using common approaches and protocols across multiple sites. However, insufficient research has been done on the topic, and there is no consensus on how to account for this effect to get unbiased measurements of soil CO_2 effluxes. Thus it is currently unknown what effect this bias have on up scaled annual soil CO_2 efflux estimates for different ecosystems, and how unbiased measurements can be performed during low u_*.

25 Our study had two aims. The first aim was to quantify the effect of u_* on automated closed chamber soil CO_2 effluxes in the short-term (i.e. effect on diurnal fluxes) and in the long-term (i.e. effect on annual estimates of CO_2 efflux). The second aim was to test the hypothesis that the overestimation of soil CO_2 effluxes during low u_* was due to insufficient mixing of the open air above the soil surface and to test if unbiased soil CO_2 efflux measurements could be achieved during low u_* by artificially inducing mixing of the air around the soil chambers by a fan.

2 Materials and Methods

2.1 Site description

Measurements were performed at the Danish ICOS RI site DK-Sor at 40 m a.s.l. (55°29'13' N, 55°38'45' E). Measurements of tower-based eddy-covariance have been running since 1996. The climate is temperate maritime with an annual average temperature of 8.5 °C and an annual average precipitation of 564 mm (Pilegaard et al. 2011).

A dense forest covers the site, and the dominant tree species is European beech (*Fagus sylvatica* L.) with scattered stands of conifers such as Norway spruce (*Picea abies* (L.) Karst) and larch (*Larix decidua* Mill.) constituting 20 % of the forest (Wu et al. 2013). The stand of beech around the flux tower was planted in 1921 and had an average height of 28 m and an average diameter at breast height of 42 cm in 2010. The average annual duration of canopy cover is 180 days with a peak LAI of 5.0, and the tree stem density is 266 per hectare. The understory is poorly developed due to the well-developed canopy, causing a sparsely vegetated forest floor. During spring, however, part of the forest floor is covered by wood anemone (*Anemone nemorosa* L.).

The soil is classified as alfisols or mollisols (depending on the base saturation) with an organic layer with a depth of 10-40 cm. The soil carbon pool (down to a depth of 1 m) is 20 kg m^{-2}, with a C/N ratio of about 20 in the upper organic soil layers, dropping to about 10 in the lower mineral layers (Østergård, 2001).

2.2 One year campaign of soil CO_2 efflux and friction velocity measurements

Soil CO_2 efflux was measured automatically over one year from 10 October 2014 to 30 September 2015 (356 days) with five 8100-104 Long-Term CO_2 flux chambers and three 8100-101 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). Measurements were made on permanent, circular soil collars (20 cm diameter) that were inserted 4 cm into the soil prior to the measurement period. The average soil collar height was 5.2 cm with the individual collar heights ranging from 4.5 to 5.8 cm. The automated chambers each measured soil CO_2 effluxes once every hour and were positioned within 15 m of the flux tower. Soil CO_2 efflux was also measured manually at 12 additional circular plots with soil collars (10 cm diameter) installed 4 cm into the soil as for the automated chambers. The manual plots were positioned close to the automated chamber plots (< 10 meters) and also within 15 m of the flux tower. Soil CO_2 efflux at the 12 plots were each measured every two weeks during day-time between 09:00–15:00 CET on days with little or no rain using a portable 8100-102 10 cm survey chamber connected to a LI-8100A Automated Soil CO_2 Flux System (LI-COR Environmental, Lincoln, Nebraska USA). Both plots for automated and manual measurements contained bare forest floor including litter but no shrubs or saplings.

There were three gaps in the automatic data collection. From 12 November to 22 November an unknown system failure occurred. From 3 February to 10 February measurements were stopped to prevent damage to the system due to accumulated snow. From 19 May to 23 July the system did not run due to a mechanical system failure, which required repair. This led to a data coverage of 76 % of the days (272 days out of 356 days). Chamber closure time was 90 and 150 s for the automated and

manual chambers, respectively, and the first 20 seconds after chamber closure was discarded (the dead band). Soil temperature and soil moisture content were measured at a depth of 5 cm for both the manual and automated measurements. For the automated measurements, 6 soil thermometers were distributed close to the soil chambers, such that no soil chamber was further away than 10 m from an individual soil sensor.

5 Alongside the chamber measurements, wind speed in three dimensions was measured by a sonic anemometer (HS-50 Research Anemometer, Gill Instruments Limited, Lymington, UK) at a height of 43 m above soil surface on the flux tower from which u_* was calculated according to Stull (1988).

2.3 Fan experiment campaign

During a 20 day campaign in July and August 2016 soil CO_2 effluxes were measured at the site with four of the 8100-104
10 Long-Term CO_2 flux chambers and two of the 8100-101 Long-Term CO_2 flux chambers, with each chamber measuring soil CO_2 effluxes every two hours using a chamber closure time of 5 minutes. A dead band of 60 s, longer than the 20 s used for the one year campaign, was required, as well as a longer closure time, because an external gas analyser was attached to the LI-8100A during the fan experiment. The longer chamber closure time was used because the external analyser required a larger difference in CO_2 concentration during chamber measurements to achieve sufficient precision. The longer dead band
15 was used because the extra volume added to the system by the external gas analyser caused longer response times and therefore also longer time to achieve stability after chamber closure. The aim of the campaign was to test if artificially increasing the mixing of air around the soil chamber would eliminate the bias of low u_* on measured chamber soil CO_2 effluxes. The artificial air mixing for each chamber was provided by 30 cm diameter table fans facing the chamber (Model 546601, HP Schou A/S, Kolding, Denmark) positioned 3 m from the soil chamber and at a height of 30 cm from the soil
20 surface to the middle of the fan. The fans provided a wind speed of 1.2-1.5 m s^{-1} at the chamber collars. During the first 10 days of the campaign, fans were installed at three chambers, resulting in three chambers with artificial air mixing and three chambers experiencing ambient conditions. During the last 10 days of the campaign the fans were moved to the other three chambers, thus providing a data set with 10 days ambient and 10 days with artificial air mixing on all six chambers. u_* at a height of 43 m above the soil surface was calculated similarly to the one year campaign.

25 ## 2.4 Data analysis

All data analysis was done using R (R Core Team, 2014). Because the current manuscript focuses on the potential error of low turbulent air mixing and because fluxes calculated using non-linear regression fitting may add additional aspects of uncertainty to the calculated fluxes, we focused on CO_2 effluxes calculated on a time and area basis by applying linear regression to the increase in chamber CO_2 concentration during chamber closure time. However, non-linear effluxes were
30 calculated as well, and at four different dead bands of 10, 20, 30 and 40 s (see supplementary information). The linear fluxes were calculated in R (R Core Team, 2014) using the lm function and the non-linear fluxes were calculated by fitting the non-linear equation suggested by Hutchinson and Mosier (1981) with the nlsLM function (minpack.lm package) for model fitting

in R. Quality control for the automated chamber effluxes was done by removing fluxes with an $r^2 < 0.95$ of the linear regression before further analysis, equal to 17 % of the measurements for the one year campaign and 1 % of the measurements for the fan experiment campaign. For the manual measurements, the quality control was done in the field directly following a measurement. If the coefficient of variance of the flux (as provided by the LI-COR software immediately following a measurement) was higher than 1.4, the measurement was discarded and an extra measurement was performed on the soil collar.

The calculated effluxes for the one year campaign were paired with u_* data from the eddy covariance system in the mast (43 m). The u_* values were used to create sub-datasets by a u_* threshold filtering technique, where effluxes measured at u_* values lower than a specific threshold value, had been filtered out and removed from the dataset (Aubinet et al. 2000). 12 different u_* threshold values were used, ranging from 0.1 to 1.2 m s^{-1}, with a successive higher u_* threshold value of 0.1 m s^{-1}. Thus, 12 different sub-datasets each with a specific u_* threshold value were derived from the one year campaign soil CO_2 effluxes. The number of soil CO_2 effluxes was 43505 for the unfiltered dataset. For the 12 different sub-datasets going from a u_* threshold value of 0.1 to 1.2 m s^{-1}, the number of soil CO_2 effluxes was 32966, 26848, 22185, 18557, 15787, 13449, 11472, 9533, 7950, 6571, 5481 and 4571, respectively.

For each of the sub-datasets, diurnal ensemble averages of soil CO_2 efflux were calculated for each of the four distinct seasons at the site. Summer: July and August, autumn: September, October and November, spring: March, April and May and winter: December, January and February.

The annual soil CO_2 efflux was obtained for each sub-dataset from the mean soil CO_2 efflux for each hour of the day for each month. From this a daily mean was calculated for each month. Monthly soil CO_2 effluxes were calculated as the sum of the daily soil CO_2 efflux in the respective month and the annual soil CO_2 efflux was calculated. One period of data outage due to system failure (20 May to 22 June) was gap filled by linear interpolation between hourly values of mean diurnal patterns that were calculated from the adjacent periods of the data gap.

In addition to the annual soil CO_2 effluxes using all 24 hours throughout the day, the day-time annual soil CO_2 efflux was calculated in a similar manner, except that only measurements made between 09:00–15:00 CET were used.

The manually measured soil CO_2 effluxes were used to parameterize the empirical model

$$R_s = R_{283} \exp\left[-E_0\left(\frac{1}{T_s+273.15-T_0}-\frac{1}{T_s-T_0}\right)\right], \tag{1}$$

of Lloyd and Taylor (1994), where T_s is soil temperature at 5 cm depth and R_{283} is the base respiration at a soil temperature of 10 °C. T_0 and E_0 are fitted parameters. The model was fitted using nonlinear least squares regression based on a Levenberg–Marquardt algorithm using nlsLM in the R package minpack.lm (Elzhov et al. 2015). The model was used to form a continuous time series of mean daily soil CO_2 effluxes throughout the one year by using a continuous measurement of soil temperature measured at 5 cm depth at the site as input to the model. From the modelled daily soil CO_2 effluxes, monthly soil CO_2 effluxes were calculated as the sum of the daily soil CO_2 effluxes in the respective month. From this the annual soil CO_2 efflux was calculated as the sum of all the 12 monthly CO_2 effluxes.

3 Results

3.1 Friction velocity and soil CO_2 effluxes

Soil CO_2 efflux generally exhibited a diurnal pattern inversely related to the diurnal pattern of u_*, with the highest effluxes seen during night-time, when u_* was lowest (Fig. 1). During summer, autumn and spring, u_* showed a clear diurnal pattern

5 with highest values during day-time and lowest values during night-time (Fig. 2a, b, d), while this pattern was weaker during winter (Fig. 2c).

Average hourly soil temperature for the automated soil chambers measured at 5 cm depth showed no diurnality during winter (Fig. 3c). However, for summer, autumn and spring a slight diurnal pattern was observed with lowest temperatures at 7–10 CET and highest temperatures late in the afternoon or early in the evening (Fig. 3a, b, d).

10 When comparing the soil CO_2 effluxes measured with the automated chambers during the one year campaign with u_*, we found a clear relationship with higher soil CO_2 effluxes at lower u_* values (Fig. 4), i.e. a significant negative correlation between u_* and soil CO_2 efflux for all seasons (P= < 0.001, r^2= 0.065). The relationship seemed, however, to level off at a u_* threshold value of approximately 0.7 m s^{-1}. Further increasing the u_* threshold value only led to a small or no further decrease in estimated effluxes.

15 Based on this result, we then calculated the mean diurnal pattern of soil CO_2 efflux for each season during the one year campaign at different u_* threshold values (Fig. 5). With no u_* filtering, the soil CO_2 efflux showed a clear diurnal pattern across all seasons with highest effluxes during night-time. This was inversely related to the diurnal pattern of u_* (Fig. 2). The difference between night-time and day-time was most pronounced during summer, where night-time (21–3 CET) effluxes were 35 % higher than day-time (9–15 CET) effluxes. Applying a successively higher u_* threshold value decreased the

20 difference between day-time and night-time effluxes for all seasons. The most dramatic effect was seen between no u_* threshold value to a value of 0.3 m s^{-1} and 0.5 m s^{-1}. Increasing the u_* threshold value from 0.5 m s^{-1} to 0.7 m s^{-1} only led to a slight change in the diurnal pattern. The u_* filtering acted primarily by lowering the high night-time effluxes and only by slightly lowering the day-time effluxes. This uneven lowering of effluxes across the day changed the distinct diurnal pattern with a large difference between night-time and day-time effluxes, i.e. at a u_* threshold value of 0.5 m s^{-1} and 0.7 m s^{-1}, the

25 diurnal pattern of soil CO_2 efflux for summer was more uniform across the day, with only slightly lower effluxes in the afternoon. The change in the diurnal pattern in response to an increased u_* threshold value was also seen for the other seasons. For winter and spring, the diurnal pattern of soil CO_2 efflux became more uniform, with no apparent difference between night-time and day-time fluxes (Fig. 5k to 5o and 5p to 5t, respectively). For autumn, applying a u_* filtering procedure reversed the diurnal pattern from the highest effluxes being seen during night-time, to the highest effluxes being

30 seem during day-time.

Looking at mean daily soil CO_2 effluxes from the automated chambers revealed that they generally followed the soil temperature throughout the year (Fig. 6a). The same was found for the soil CO_2 effluxes based on the manual chamber measurements and the output of the empirical model (Fig. 6c). A high day to day variability was found throughout the year

for the automated measurements, which was especially pronounced during summer. Although following the same seasonal pattern, the modelled effluxes based on the manual measurements were slightly lower than those from the automated measurements (summer: 2.97 for the manual vs. 4.10 μmol m^{-2} s^{-1} for the automated measurements, autumn: 2.36 for the manual vs. 2.86 μmol m^{-2} s^{-1} for the automated measurements, winter: 0.485 for the manual vs. 0.608 μmol m^{-2} s^{-1} for the automated measurements) except for spring where the manual measurements were the highest (1.19 for the manual vs. 1.04 μmol m^{-2} s^{-1} for the automated measurements). Applying a successively higher u_* threshold value to the automated soil CO_2 effluxes generally decreased the daily mean soil CO_2 effluxes, as well as the day to day variability (Fig. 6b). The biggest decrease was seen between no u_* filter to a u_* threshold value of 0.7 m s^{-1}. Further increasing the u_* threshold value only led to minor additional decreases in the mean daily soil CO_2 effluxes. At a u_* threshold value of 0.7 m s^{-1} the mean daily soil CO_2 effluxes for summer, autumn, winter and spring was lowered with 25 % (to 3.09 μmol m^{-2} s^{-1}), 17 % (to 2.37 μmol m^{-2} s^{-1}), 19 % (to 0.492 μmol m^{-2} s^{-1}) and 18 % (to 0.856 μmol m^{-2} s^{-1}) respectively in comparison to when no u_* filter was applied.

3.2 Annual soil CO_2 efflux

Annual soil CO_2 effluxes, based on the automated chamber flux data for the one year campaign, were calculated with the exclusion of data at different u_* threshold values (Fig. 7). The highest annual soil CO_2 efflux was found when no u_* filter was used (808.9 g C m^{-2} yr^{-1}). Increasing the u_* threshold value decreased the annual soil CO_2 efflux, with the largest decrease of 21 % observed between unfiltered data to a u_* threshold value of 0.7 m s^{-1} (from 808.9 to 641.7 g C m^{-2} yr^{-1}). Between a u_* threshold value of 0.7 to 1.2 m s^{-1}, only a small decrease in annual soil CO_2 efflux of 7 % was seen (from 641.7 to 596.9 g C m^{-2} yr^{-1}). The annual soil CO_2 efflux from the empirical model based on the manual chamber measurements was 666.6 g C m^{-2} yr^{-1}.

3.3 Day-time soil CO_2 effluxes vs. daily effluxes

To assess the consequences of using only day-time soil CO_2 efflux data, instead of data for the entire day, when upscaling to annual soil CO_2 efflux, we calculated annual soil CO_2 effluxes for the one year campaign at different u_* threshold values using only fluxes measured between 9–15 CET, and compared these to the annual soil CO_2 effluxes calculated using effluxes for the entire day at different u_* threshold values (see section 3.2).

At no u_* filter, the annual day-time soil CO_2 efflux was 13 % lower than the annual entire day soil CO_2 efflux (808.9 vs. 703.3 g C m^{-2} yr^{-1} for the annual entire day and day-time soil CO_2 effluxes respectively. Fig. 8). Increasing the u_* threshold value decreased the difference between annual day-time soil CO_2 effluxes and entire day effluxes, with a steep decrease from 13 to 4.5 % observed between no u_* filtering to a u_* threshold value of 0.3 m s^{-1} (705.4 vs. 673.7 g C m^{-2} yr^{-1} for the annual entire day and day-time soil CO_2 effluxes respectively). Further increasing the u_* threshold value only resulted in a minor change in the relationship between the annual day-time soil CO_2 efflux and the entire day efflux. However, they were almost

identical at a u_* threshold value of 1.2 m s^{-1} (596.9 vs. 595.5 g C m^{-2} yr^{-1} for the annual entire day and day-time soil CO_2 effluxes respectively).

3.4 Fan experiment campaign

The observation that u_* filtering profoundly affected estimated soil CO_2 effluxes led us to design a simple fan experiment to test if the bias of low atmospheric turbulent mixing on the measured chamber soil CO_2 effluxes could be eliminated by ensuring adequate mixing of air around the soil chamber. When no fan was installed, a significant negative relationship was found between soil CO_2 efflux and u_* (r^2 = 0.040, P = < 0.001, slope = -0.377, data now shown) comparable to the one year campaign (Fig. 4). However, with fans installed the negative relationship changed into a significant positive relationship (r^2 = 0.080, P = < 0.001, slope = 0.353), clearly indicating a strong effect of installing fans. With no fans, the soil CO_2 efflux showed a clear diurnal pattern with highest effluxes during night-time (Fig. 9a and 9b). However with fans, the opposite diurnal pattern with highest effluxes during day-time was seen. The change in diurnal pattern when using a fan was primarily due to a decrease of the high night-time effluxes (50 % lower for effluxes measured at 21–03 CET). However, a decrease in day-time effluxes was also observed when using the fans (26 % lower for effluxes measured at 9–15 CET).

4 Discussion

4.1 Friction velocity and soil CO_2 effluxes

Soil CO_2 effluxes generally followed the seasonal changes in soil temperature, being highest in summer and lowest in winter. Our one year measurement series with a total of 52131 individual soil CO_2 efflux measurements from 8 automatic chambers clearly showed a negative relationship with the level of atmospheric turbulence (u_*), which was especially visible during periods with similar soil temperature, where apparent rates of soil CO_2 effluxes were clearly higher at lower compared to higher u_* values.

For closed chamber measurements to represent real R_s, we assume steady-state diffusion from the source in the soil to the atmosphere according to Fick's law. Accordingly, there must be a constant concentration of atmospheric CO_2, for the flux to be stable, and the physical application of a closed chamber should not change or break down any CO_2 gradients. Otherwise advection may take place. Our data strongly indicate that these assumptions are likely not met during low u_*, when the high soil CO_2 effluxes were observed.

u_* typically varies on a daily basis with low u_* during calm nights and high u_* during daytime (Stull, 1988). This is in agreement with our results (Fig. 2). The low atmospheric turbulence during calm nights has been found 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). This lowers the concentration gradient of CO_2 from the soil to the atmosphere causing a build-up of CO_2 in the soil (Wohlfahrt et al., 2005; Flechard et al., 2007). This acts to supress the apparent soil CO_2 efflux, and may also lead to a higher apparent soil CO_2

efflux once turbulence is re-established, because of the subsequent release of the CO_2 that has been build up in the soil (Massman et al., 1997). Our chamber measurements, however, show higher and not lower soil CO_2 effluxes during low u_*. It is unlikely that u_* has a direct effect on the biological activity of bacteria, fungi and/or plant roots in the soil and thus it seems evident that the apparent increase in soil CO_2 efflux measured during low u_* is a measurement bias of the closed chamber technique. Only a few studies have demonstrated the potential problem of using closed chambers during periods with low atmospheric turbulence (Görres et al., 2016; Koskinen et al., 2014; Lai et al., 2012; Schneider et al., 2009). Görres et al. (2016) suggested that during low atmospheric turbulence, the chamber causes a disturbance and/or mixing of the stratified layer of still air above the soil surface, as the chamber moves on to the soil at the beginning of a measurement, while Lai et al. (2012) accredited the error to be caused by a similar effect by the internal chamber fan. In both cases, the CO_2 rich air just above the soil surface is instantly mixed with less CO_2 rich air from above. This causes a sudden drop in the CO_2 concentration just above the soil surface, which in turn increases the concentration gradient between the soil and the atmosphere, thus leading to an apparent high CO_2 efflux being measured by the chamber (Görres et al., 2016). Our results support this hypothesis. In addition, we observed that soil temperatures were similar in the data sets with different u_* threshold values (data not shown), indicating that the relationship between flux rates and u_* were not confounded by such potential differences in the different subsets of observations. We also showed that this error of overestimation of soil CO_2 effluxes during low u_* had a dramatic effect on both the diurnal pattern of soil CO_2 efflux, and on the estimate of annual soil CO_2 efflux, as we will discuss this in the following sections. An alternative and/or additional reason for the stable conditions above the soil surface during calm periods may be the stratification of the air caused by cooling due to longwave net radiation loss from the soil as suggested by Riederer et al. (2014). This aspect was, however, not addressed in the current study.

4.2 Diurnal pattern of soil CO_2 effluxes

The hourly measurements of soil CO_2 effluxes showed a clear diurnal pattern, with generally highest effluxes during night-time, when no u_* filter was applied to the data (Fig. 5a, 5f, 5k, 5p). Soil temperature at 5 cm depth, however, did not show a diurnal pattern for winter, and only a slight diurnal pattern for the other seasons with highest temperatures late in the afternoon or early in the evening (Fig. 3). This could indicate a hysteresis between soil CO_2 efflux and soil temperature for summer, autumn and spring. A few studies with automated closed chambers have found a similar hysteresis between soil CO_2 efflux and soil temperature (e.g. Phillips et al., 2011; Savage et al., 2013; Tang and Baldocchi, 2005). The hysteresis has been explained as a result of priming of the soil bacteria by carbon exudates from plant roots (Kuzyakov and Gavrichkova, 2010). During day-time in the growing season, plants assimilate carbon via photosynthesis. Part of the assimilated carbon go to the roots via the phloem, and is released into the rhizosphere (Kuzyakov, 2002). Once in the soil, this carbon is readily consumed by bacteria leading to an increase in R_s. Even though photosynthesis takes place during day-time, the increase in R_s has been found to lag after photosynthesis, because the photo assimilates need to be transported from the leaves to the roots via the phloem. Varying lag times have been found, with the shortest times for short plants such as grasses, and lag

times of up to 4–5 days for mature trees (Kuzyakov and Gavrichkova, 2010). However, when applying a successively higher u_* threshold value, the diurnal patterns of soil CO_2 efflux across all seasons changed, mostly due to removal of the overestimated night-time effluxes. For our measurements, the overestimation of chamber effluxes due to low u_* thus works as a selective systematic error that mostly applies to night-time of the diurnal pattern. This is in agreement with other studies

5   (Görres et al., 2016; Koskinen et al., 2014; Lai et al., 2012; Schneider et al., 2009). During summer, we observed that the difference between night-time and day-time was smaller than during spring and autumn, and only the fluxes between 10–20 CET were slightly lower than the night-time fluxes. It is possible that this pattern is due to an increase in the night-time soil respiration due to priming of the soil organisms via carbon flow from the roots into the rhizosphere and soil. This was, however, not tested in the present study. For the other seasons priming is not as likely, since only low or no photosynthesis

10   takes place during this time. During winter and spring, the diurnal patterns changed from a clear diurnal pattern with highest night-time fluxes to no difference between night and day-time fluxes when applying the u_* filtering. During autumn, it changed from a clear diurnal pattern with highest night-time fluxes, to a pattern where the fluxes seem to be slightly higher at daytime.

Our results show that overestimation of soil CO_2 effluxes at low u_* can change the apparent diurnal pattern of soil CO_2

15   effluxes, and they highlight the importance of taking this source of error into account, since negligence of the problem may lead to misinterpretation of the relationship between R_s and its physical drivers like temperature and soil humidity, as well as lead to erroneous estimation of lag times between R_s rates and flow of carbon from recent plant assimilates. Much research has been done on time lags between inputs of carbon via photosynthesis and R_s using closed chambers (Högberg et al., 2001; Tang et al., 2005). We expect that it is possible that overestimates of soil CO_2 effluxes at low u_* may have influenced the

20   interpretation of the diurnal pattern in some previous studies that have not taken overestimation of effluxes during low u_* into account. It could therefore be valuable to re-evaluate these studies by including data of u_* if such data are available.

4.3 Annual soil CO_2 effluxes

The u_* filtering had a considerable effect on the annual soil CO_2 efflux estimates based on the automated chamber measurements by decreasing the annual efflux in response to increasing the u_* threshold value. This could be expected from

25   the observed negative relationship between soil CO_2 efflux and u_*. The annual soil CO_2 efflux modelled from the manual day-time-only chamber measurements measured at 12 soil collars within 10 meters from the automated chambers was 666.6 g C m^{-2} yr^{-1}. During day-time (9–15 CET) u_* was usually high (Fig. 2). The risk of overestimation of a fraction of the manual soil CO_2 efflux measurements due to low u_* can therefore be viewed as minor. The automated chamber annual soil CO_2 efflux including all data was 808.9 g C m^{-2} yr^{-1}, i.e. a value much higher than the 666.6 g C m^{-2} yr^{-1} from the manual

30   chambers. However, the annual soil CO_2 efflux estimate from the automated chamber measurements decreased to 663.4 g C m^{-2} yr^{-1} and 596.8 g C m^{-2} yr^{-1} at a u_* threshold value of 0.5 m s^{-1} and 1.2 m s^{-1}, respectively, i.e. much closer to the annual soil CO_2 efflux from the manual measurements (Fig. 7). Thus, it seems that the automated measurement and the manual day-

time measurements provide comparable estimates of annual soil CO_2 effluxes, when the effect of low u_* is accounted for, as part of the quality checking procedure for the automated measurements. This seems to increase the confidence in the much less frequent manual measurements, and potentially shows that day-time only measurements, at least in our case, was not a major source of error for the upscaling to an annual estimate of soil CO_2 efflux. This is in agreement with Juszczak et al.

5 (2012) that found that there was no difference between day-time and night-time effluxes when the correct u_* threshold value had been applied. To exemplify this, we compared the annual soil CO_2 effluxes from the automated chambers with estimates of annual soil CO_2 effluxes based on only day-time (9–15 CET) soil CO_2 effluxes from the automated chambers. The day-time annual soil CO_2 efflux was 13 % lower than the annual entire day soil CO_2 efflux with no u_* filter, and decreased to 4.5 % at a u_* filter of 0.3 m s^{-1} (Fig. 8) i.e. resulting in more comparable annual soil CO_2 effluxes when the overestimation of u_*

10 is accounted for.

4.4 Fan experiment

Mixing of the air around the soil chambers with fans had a considerable effect by decreasing the measured soil CO_2 effluxes (Fig. 9). The biggest difference was seen during night-time, but even at day-time a smaller effect was observed. This selective lowering of effluxes changed the apparent diurnal pattern, such that the highest fluxes now were measured during

15 day-time. By using a fan we hypothesised that the effect of breaking the stratified layer by the chamber during low u_* would not occur because the artificially induced wind by the fan continuously ensured proper mixing of the air around the chamber and thus likely prevented a build-up of a stratified layer of CO_2. We believe 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 proposed mechanism for the apparent higher soil CO_2 efflux at low u_* can no longer take place, due to mixing of the air by the fan prior to the measurement. Thus

20 chamber soil CO_2 measurements are no longer overestimated at low u_*, which is also seen by that there was no negative relationship between soil CO_2 effluxes and u_* when a fan was used. However, when using the fans we also observed an apparent decrease in the measured day-time soil CO_2 effluxes, when u_* was generally high. We suggest three potential causes of this difference in the day-time effluxes. Firstly, the fan experiment campaign ran over 20 days, i.e. 10 of the days were with a fan installed and 10 days were without a fan. Thus a comparison for a chamber is made at different times, and

25 differences in environmental conditions may have caused differences in the rates of R_s between the two periods. Indeed, a difference in the mean soil temperature at 5 cm depth of 1 °C was found between the two periods. However, the difference in soil CO_2 effluxes was found for both groups of chambers, i.e. both for the ones with a fan installed during the cold period and for those with a fan installed during the warm period, and thus the potential directional bias of differences in soil temperature during the two periods can be ruled out. Secondly, the slight decrease of day-time effluxes when fans were

30 installed could be because low u_* was sometimes observed also during day-time, although to a much lesser degree than during night-time. A similar small decrease of the day-time effluxes for the one year campaign when increasing the u_* threshold value was also observed (Fig. 5), highlighting that there may still be such an effect even during day-time. Thirdly,

it is possible that although a fan eliminated the bias of low u_* on chamber effluxes, the wind induced by the fan introduced another potential bias to the measurement. Steady-state diffusion rate of CO_2 out of the soil with and without a chamber is a requirement for unbiased chamber measurements of soil CO_2 efflux. However, the rate of diffusion of a gas out of the soil is known to be highly sensitive to wind speed at the soil surface, and higher wind speeds can increase the diffusion rate or even

5   cause advective transport out of the soil (Janssens et al., 2000; Roland et al., 2015). This effect is well-known to cause severe overestimation of soil CO_2 effluxes when soil chambers are equipped with a heavy internal fan because the resulting wind speed inside the chamber is much higher than outside (e.g. Hanson et al., 1993; Hooper et al., 2002; Le Dantec et al., 1999). The LI-8100A chambers used in our experiments do not have an internal fan. Instead they rely on the air movement that is created when air is pumped to and from the chamber in combination with the spherical shape of the chamber for adequate

10   mixing of chamber air (LI-COR Biosciences, 2015). The wind speed in the LI-8100A chamber may therefore be fairly low compared to outside conditions. 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 a lower rate of diffusion and consequently a lower measured soil CO_2 efflux, and that this effect increases with the installation of fans that increase the wind speed outside the chamber. A similar effect has also been found to occur during natural conditions outside the chamber

15   where soil effluxes measured by micrometeorological methods have been found to increase with increasing ambient wind speed (Denmead and Reicosky, 2003). To eliminate this potential bias due to difference in wind speed, a closer matching of the chamber wind speed with the ambient wind speed can be attempted, as suggested by Rochette and Hutchinson (2005). It is possible that a lower wind speed induced by a fan would be adequate to eliminate the effect of low u_*. This would potentially lower the difference in wind between the outside and inside of the chamber, thus minimizing the potential bias

20   due to different wind speeds. This was, however, not tested in the current study.

4.5 Towards unbiased soil CO_2 effluxes

Chamber-based measurements of soil efflux of CO_2 and other greenhouse gases play an important role in constraining the global carbon cycle. Thus it is crucial to obtain unbiased measurements that are as close to the true rates of emissions or uptake as possible. Many biases have already been quantified or even eliminated through methodological and technical

25   advances. Here we showed that the bias of soil CO_2 efflux measurements at low u_* can be accounted for by using a u_* filtering procedure. However, we do see a number of challenges with this methodology that needs to be addressed. One challenge is to choose the correct u_* threshold value. A similar challenge has faced the eddy covariance method, where multiple methods have been developed to determine the right u_* threshold value (Gu et al., 2005). One method is to subjectively choose the appropriate level e.g. based on visual inspection of scatter plots of night-time fluxes versus u_* (Gu et

30   al., 2005). For our data, this would mean choosing a u_* threshold value, where further increasing the u_* threshold value would no longer change the mean soil CO_2 effluxes observed. When looking at the diurnal patterns in Fig. 5, only a small further change in the diurnal pattern is seen between a u_* threshold value of 0.5 to 0.7 m s^{-1}, thus indicating that a u_* threshold value between 0.5 and 0.7 m s^{-1} would be appropriate. A slightly higher u_* threshold value seems to apply for the

mean hourly soil CO_2 effluxes aggregated into groups based on u_* values (Fig. 4), where no decrease in effluxes is seen after the binned group with u_* values of 0.7–0.8 m s^{-1}. Interestingly, a similar levelling off of the decrease in the estimated annual soil CO_2 efflux in response to an increasing u_* threshold value was not seen (Fig. 7). Instead, the annual soil CO_2 efflux continued to decrease slightly even after the big decrease, that was seen up to a u_* threshold value of 0.7 m s^{-1}. This indicates

5 that effluxes are still to some extend influenced by u_* at these high levels. We analysed the data to see if the u_* filtering had an influence on soil temperature, which potentially could selectively remove higher effluxes measured at higher temperatures, leaving only measurements in colder periods at high u_* threshold values (data not shown). The results could, however, not explain the continuous decrease in soil CO_2 efflux. Thus it remains an open question why this decrease is still seen. One possible explanation is that the u_* used in this experiment was measured above the canopy at a height of 43 m. The

10 soil chambers, however, were positioned at the soil surface and it is possible that the measured turbulence at 43 m was not well coupled to u_* above the soil surface surrounding the soil chambers (Thomas and Foken, 2007). A second anemometer installed closer to the soil surface (see Thomas et al., 2013) might have provided a clearer u_* threshold value.

Another method to determine the u_* threshold value is to use objective statistical methods such as the Moving Point Test also commonly used in eddy covariance data analysis (Gu et al., 2005). We suggest testing some of these methods for

15 determination of the u_* threshold value for chamber CO_2 effluxes in future studies.

We tried to see if using a stricter flagging of data based on the r^2 value of the linear fit to the increase in CO_2 concentration during chamber closure could also be used to remove overestimated effluxes (data not shown). A similar effect of u_* on effluxes could, however, still be seen even when a higher r^2 value was used to quality check the data, indicating that even during low atmospheric turbulence the increase in chamber CO_2 concentration often followed the expected pattern. High r^2

20 values and near-linear increases in chamber CO_2 concentration during chamber deployment alone can therefore not be used as the only means of quality checking, as this may still lead to falsely accepting overestimated measurements during low turbulence.

Although we have here shown that a u_* filtering procedure seems to have a good potential to be used for quality control of closed chamber measurements of soil CO_2 efflux, it ultimately leads to data gaps that need to be filled using the appropriate

25 gap filling techniques e.g. based on established empirical relationships between measurement bias and u_* (Lai et al., 2012). Data gaps, however, equal loss of data and thus methods should be developed to be able to observe unbiased chamber measurements also during periods with low atmospheric turbulence. Valid chamber measurements during periods with low u_* are especially important since this coincide with periods of data gaps in fluxes estimated by the eddy covariance technique. Thus it is clear that chamber measurements during these periods can't be used as ground truth for the eddy

30 covariance flux estimates (Görres et al., 2016). Our results from the fan experiment have shown promising results in terms of removing the negative relationship between u_* and soil CO_2 effluxes. This is, to the best of our knowledge, the first time this has been attempted, and we expect that this method has a future potential. A few others have touched upon how to get unbiased chamber effluxes during low u_*. Lai et al. (2012) found that closed chamber CH_4 and CO_2 effluxes were

overestimated at low u_*. However, by increasing the chamber closure time to 30 minutes, they found that fluxes were no longer influenced by u_* after 13 minutes of chamber closure time, thus yielding reliable fluxes. However, it can be argued that longer chamber closure times introduce other potential biases for most closed chamber systems, because the chamber headspace concentration of CO_2 or CH_4 changes more and therefore affects the concentration gradient between soil and

5   atmosphere, which ultimately can lead to underestimation of efflux rates instead. We only used a chamber closure time of 90 seconds for the one year campaign, because this time was sufficient to adequately measure an increase in chamber headspace CO_2 concentration over time. We did, however, test if flux estimates were affected by using different dead bands of 10, 20, 30 and 40 seconds, respectively, or by using linear or non-linear flux calculation methods (see supplementary information). In all cases we observed a similar pattern of overestimation of soil CO_2 effluxes during low u_* as reported here for linearly

10 calculated effluxes with a dead-band of 20 s. Thus neither dead band nor flux calculation method could eliminate the effect of low u_* on soil CO_2 effluxes.

Even though automated closed chamber systems are based on the same principle of passive, steady-state diffusion of gases between the soil and the chamber headspace during chamber closure, they come in a wide variety of designs with various shapes and sizes. We expect that chamber design may influence the effect of u_*, although it remains to be tested. Görres et al.

15 (2016), however, also argued that automated chambers have the potential to provide unbiased chamber fluxes during low atmospheric turbulence by fulfilling certain design criteria that ensure that the stable atmospheric layer of air above the soil surface is not broken up during a measurement. These design criteria include a low chamber height of less than 20 cm and a low 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. This approach is directly in contrast with the approach we used with fans, where we ensured

20 mixing of the air above the soil surface at all times, such that any stratification was eliminated before the beginning of a measurement. The proposed design criteria may lead to a lower disturbance of the air column above the soil surface, but it is uncertain if it is possible to eliminate disturbance of air completely. One of the fundamental principles of closed chamber measurements is mixing of air inside the chamber. Even with a low chamber height we suspect that mixing of stratified air just few cm from the soil surface may lead to an overestimated flux during low u_*.

25 **5 Conclusions**

The study had two major results. Firstly, we showed that soil CO_2 effluxes measured by automated closed chambers were overestimated at low u_* throughout one year in a Danish beech forest and that this overestimation considerably biased the diurnal patterns of soil CO_2 effluxes and led to an overestimation of the annual soil CO_2 efflux. We recommend that any analysis of soil CO_2 efflux measured by automated closed chambers must consider overestimation of effluxes at low

30 atmospheric turbulence, to yield unbiased estimates of soil CO_2 effluxes. This is crucial when investigating temperature responses of R_s and biological links in ecosystems between CO_2 production and R_s on a short time scale, but also for correct estimation of annual soil CO_2 effluxes. We have shown that a u_* filtering procedure can be used to remove data that are

influenced by insufficient turbulence. The drawback of this post-processing method is, however, a loss of data and thus a loss of information during atmospheric conditions where the eddy covariance method cannot be applied. Our analysis highlights the need for methodological developments, which will allow for unbiased chamber measurements to be made also during low atmospheric turbulence. We see the results from our fan experiments as a significant step along the way. Here we

5 showed that ensuring continuous mixing of air around the soil chamber by a fan eliminated the overestimation of soil CO_2 effluxes due to low u_*, thus enabling reliable chamber measurements even at low u_*, even though the continuous mixing of air may have introduced a new bias during chamber measurements. Additional studies are needed to further explore this approach.

Acknowledgements

10 This study was funded by the free Danish Ministry for Research, Innovation and higher Education, the free Danish Research Council (DFF – 1323-00182).

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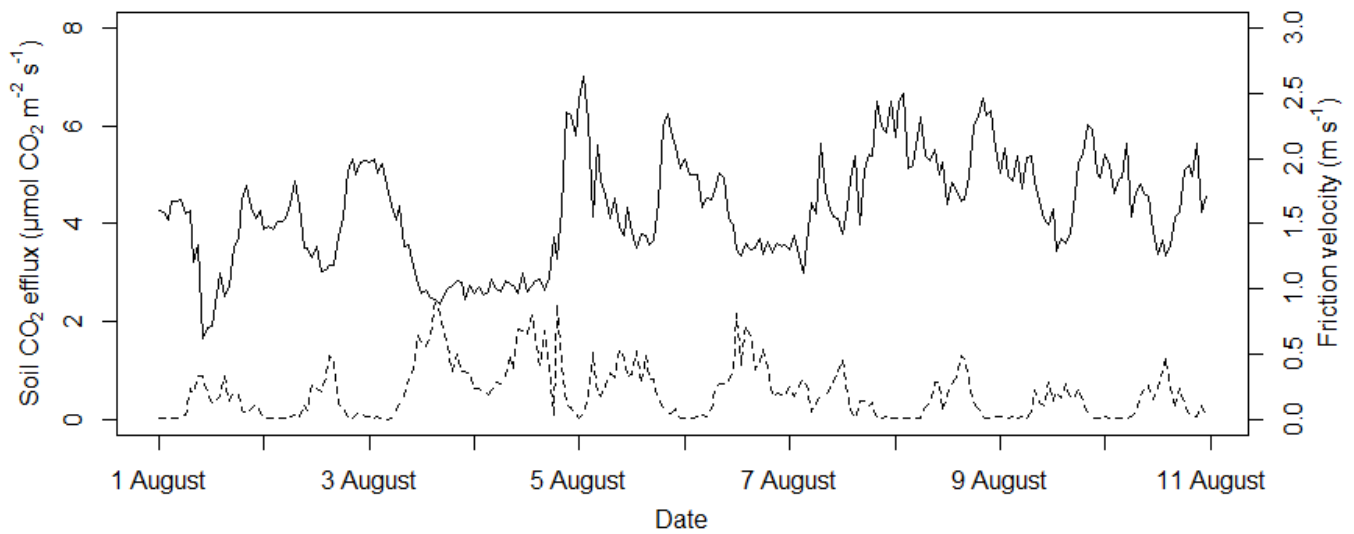
Figure 1: Example of mean hourly soil CO_2 effluxes from the eight automated chambers (solid line) and mean hourly friction velocity (u_*) at 43 m above the soil surface (dashed line) for 10 days during August 2015.
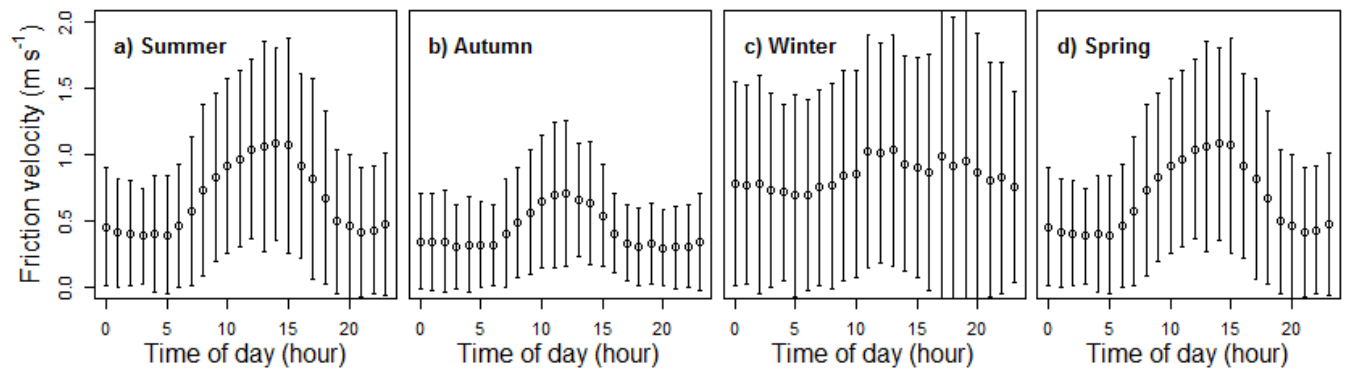
Figure 2: Mean (± standard deviation) diurnal pattern of friction velocity (u_*) at 43 m above the soil surface for summer (a), autumn (b), winter (c) and spring (d).
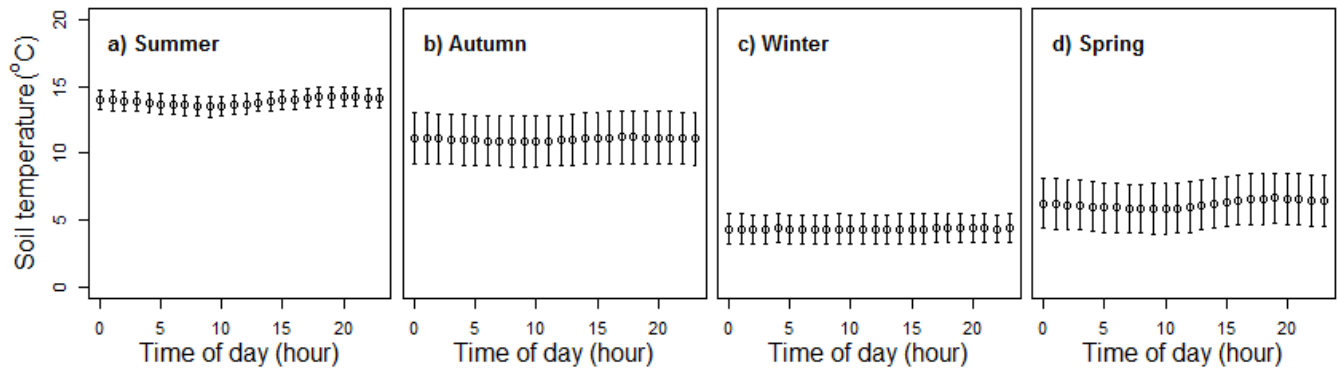
Figure 3: Seasonally averaged diurnal pattern of soil temperature (± standard deviation) at 5 cm depth measured by 6 soil thermometers close to the eight automated chambers for summer (a), autumn (b), winter (c) and spring (d).
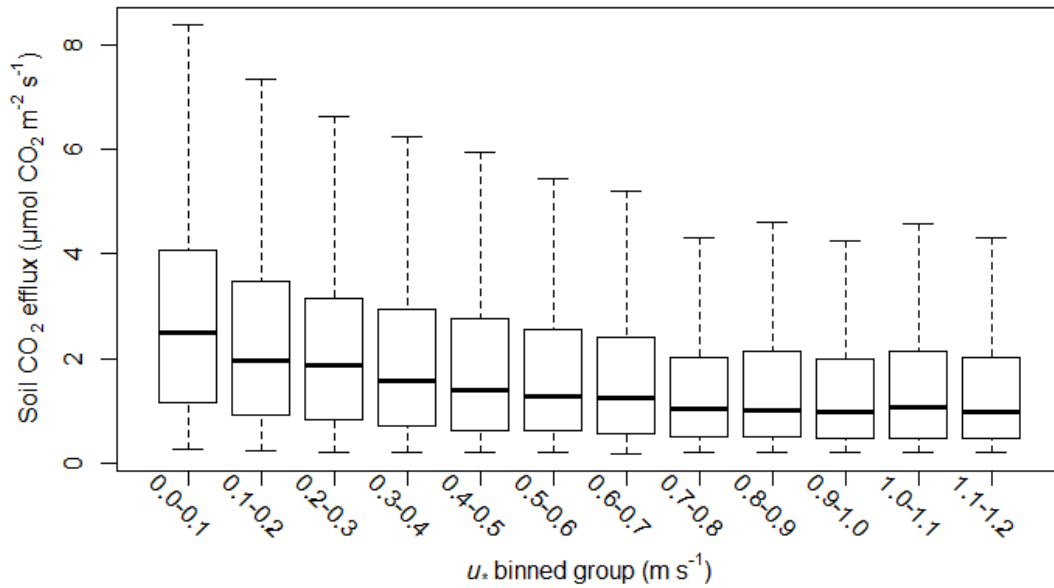
Figure 4: Boxplot of mean hourly soil CO_2 effluxes for the one year campaign plotted against the binned groups of friction velocity (u_*).
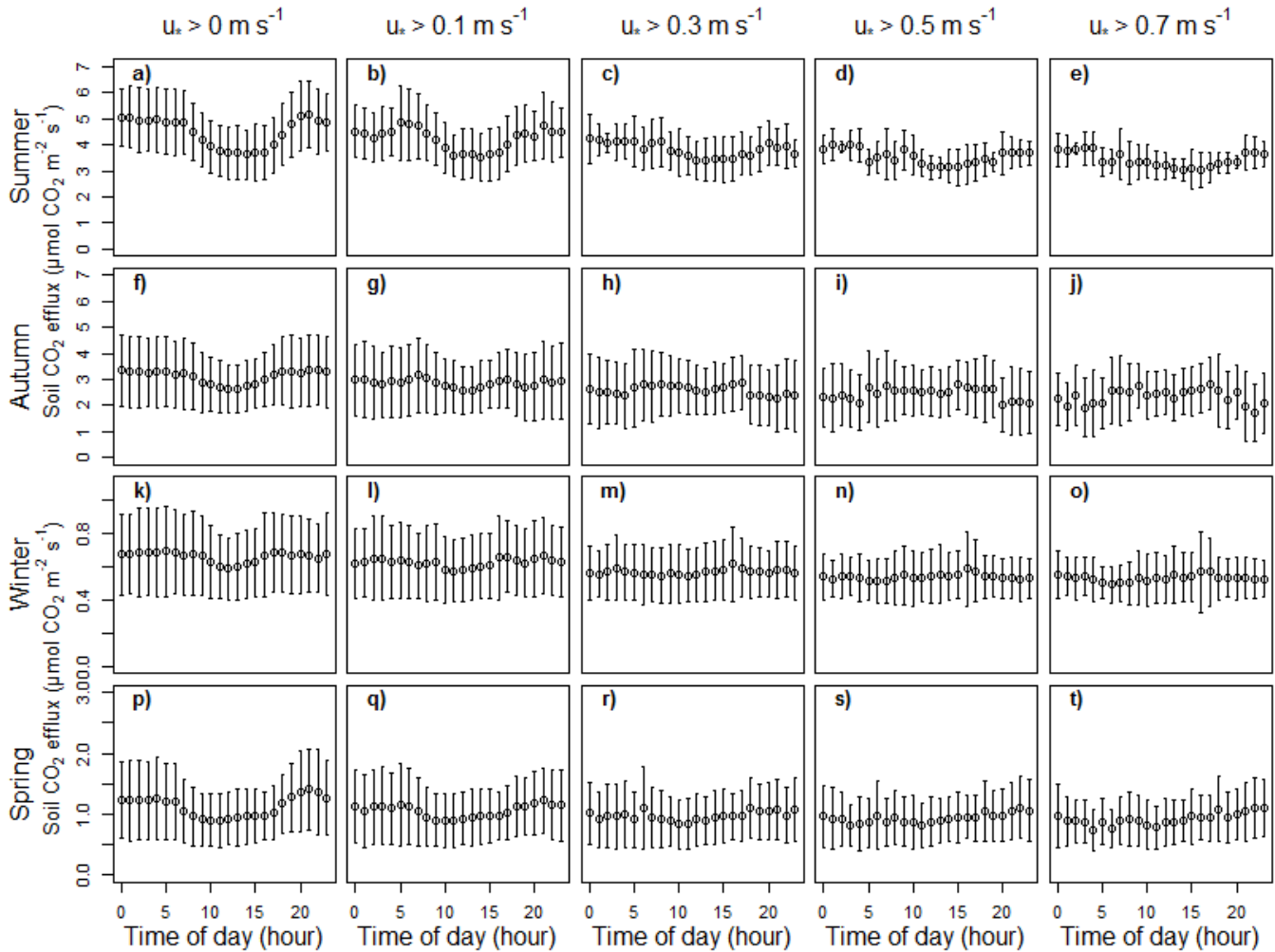
Figure 5: Seasonally averaged diurnal patterns of soil CO_2 efflux (± standard deviation), at different friction velocity (u_*) threshold values, measured by the eight automated chambers for each of the 4 seasons. From the top, the four rows show the diurnal patterns for summer, autumn, winter and spring respectively. From the left, the five collars show the diurnal 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.
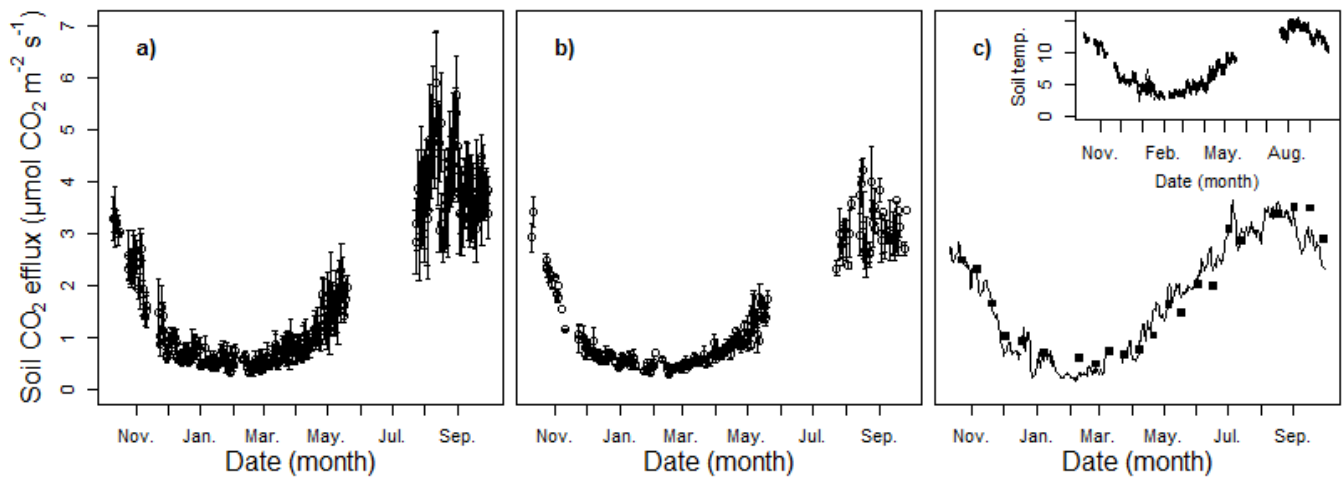
Figure 6: Comparison of the courses of the mean daily soil CO$_2$ efflux throughout the year. (a) and (b) show mean daily soil CO$_2$ effluxes (± standard deviation) throughout the year measured by the eight automated soil chambers, without friction velocity (u_*) filtering and with a u_* threshold values of 0.7 respectively. (c): The black dots show the mean soil CO$_2$ effluxes for each of the manual soil chamber campaigns, and the solid line shows the output of the empirical model based on these manual measurements. The course of soil temperature throughout the year at 5 cm depth measured at the eight automated chambers is shown on the inset in panel (c).
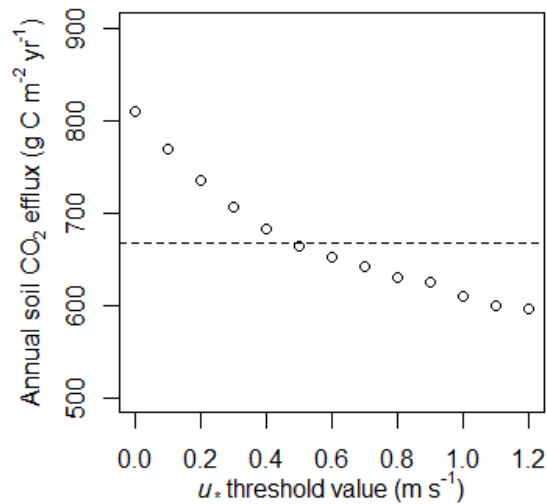
Figure 7: Estimates of annual soil CO$_2$ efflux in response to increasing the friction velocity (u_*) threshold values for the automated chamber measurements during the one year campaign. The straight dashed line shows the annual soil CO$_2$ efflux of 666.6 g C m^{-2} yr^{-1} based on the manual measurements.
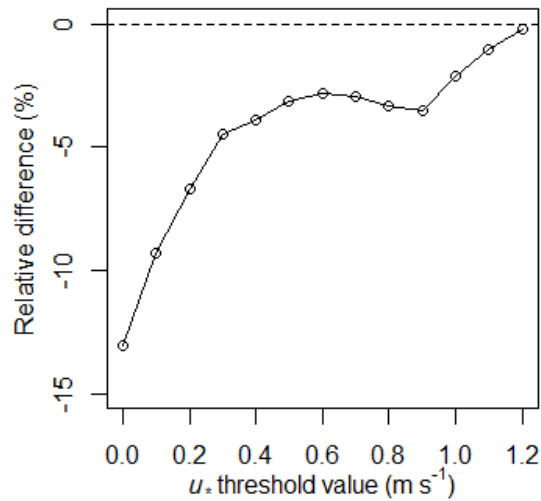
Figure 8: Relative difference between the estimates of annual soil CO$_2$ efflux based on daytime (9–15 CET) data at different friction velocity (u_*) threshold values, compared to the estimates of annual soil CO$_2$ efflux based on data for the entire day at different friction velocity (u_*) threshold values.
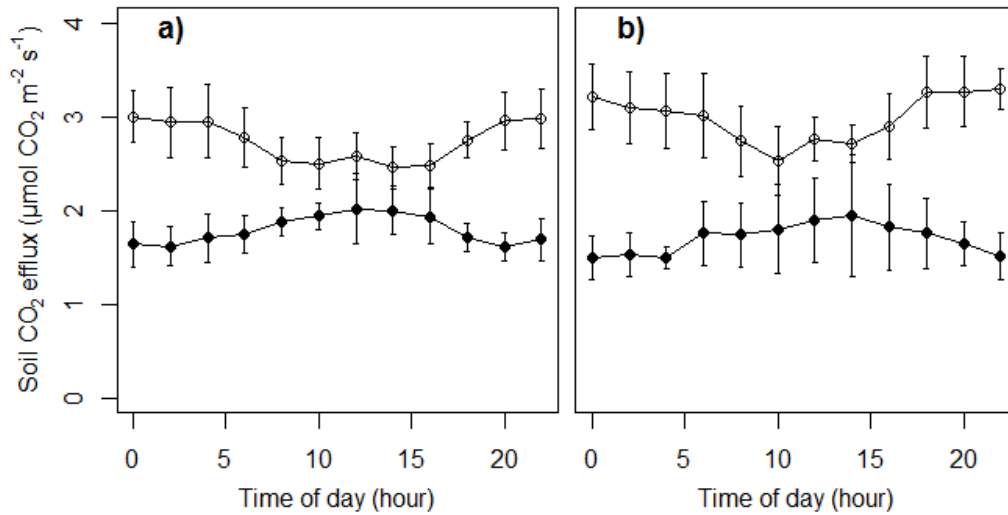
Figure 9: Diurnal pattern of soil CO$_2$ efflux, measured by the automated chambers during the fan experiment, based on bi-hourly means (± standard deviation). (a) shows the diurnal pattern for half of the chambers with and without fans, where the first 10 days were with fans (filled circles) and the last 10 days were without fans (open circles). (b) shows the diurnal 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).