How many measurements are needed to estimate accurate daily and annual soil respiration fluxes? Analysis using data from a temperate rainforest

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Abstract. Making accurate estimations of daily and annual R_s fluxes is key for understanding the carbon cycle process and
15 projecting effects of climate change. In this study we used high-frequency sampling (24-measurements per day) of R_s in a temperate rainforest during one year, with the objective of answering the questions of when and how often measurements should be made to obtain accurate estimations of daily and annual R_s. In this aim, we randomly selected data to simulate samplings of 1, 2, 4 or 6 measurements per day (distributed either during the whole day or only during daytime) combined with 4, 6, 12, 26 or 52 measurements per year. Based on the comparison of partial-data series with the full-data series, we
20 estimated the performance of different partial sampling strategies based on bias, precision and accuracy. In the case of annual R_s estimation, we compared the performance of interpolation vs. using non-linear modelling based on soil temperature. The results show that, under our study conditions, sampling twice a day was enough to accurately estimate daily R_s (RMSE<10% of average daily flux), even if both measurements were done during daytime. The highest reduction in RMSE for the estimation of annual R_s was achieved when increasing from 4 to 6 measurements per year, but reductions were
25 still relevant when further increasing the frequency of sampling. In conclusion, we found that increasing the number of field campaigns was more effective than increasing the number of measurements per day, provided a minimum of two measurements per day was used. Including nighttime measurements significantly reduced the bias and was relevant in reducing the number of field campaigns when a lower level of acceptable error (RMSE<5%) was established. Using non-linear modelling instead of linear interpolation did improve the estimation of annual R_s but not as expected. Given that most
30 of the studies of R_s use manual sampling techniques and apply only one measurement per day, we suggest making an intensive sampling at the beginning of the study to determine minimum daily and annual frequencies of sampling.

1 Introduction

Respiration is the second most important flux in ecosystems after photosynthesis, in terms of the quantities of exchange and the contribution to the total carbon cycle (Schlesinger and Andrews, 2000). Within ecosystem respiration, soil respiration (R_s) is considered a key element of the global C cycle, representing about 50-94% of the terrestrial ecosystem respiration, depending on the period of the year (Curiel Yuste et al., 2005; Goulden et al., 1996). Soil respiration is defined as the aggregation of below-ground processes of heterotrophic (microbial respiration) and autotrophic (root respiration) components (Savage et al., 2009). Accordingly, major differences in R_s are explained by the variation in metabolic activity of both autotrophic and heterotrophic components, which are driven by changes in environmental conditions (Raich and Schlesinger, 1992). This temporal heterogeneity makes the estimations of daily and annual R_s difficult, expensive and time consuming tasks. Therefore, the development of R_s measurement protocols which maximize the accuracy/measurement frequency ratio for estimating R_s will definitively accelerate the progress of our knowledge about the global carbon balance and its drivers.

Soil respiration has been reported to differ across temporal and spatial scales (Jia et al., 2006; Li et al., 2008; Vargas et al., 2010) as a result of changes in soil temperature (Lloyd and Taylor, 1994; Subke and Bahn, 2010), soil moisture (Bown et al., 2014; Gaumont-Guay et al., 2006), vegetation (Bahn et al., 2010; Buchmann, 2000), topography (Kang et al., 2003), soil texture (Dilustro et al., 2005; Pumpanen et al., 2008), and primary productivity (Bahn et al., 2010, 2008; Högberg et al., 2001; Vargas et al., 2011). Among these variables, temperature and soil moisture are the most widely used in empirical prediction models of R_s (Trumbore, 2006). This trend is consistent with the results reported by Chen et al. (2014) who, using a global database, showed that most variation in R_s was explained by mean annual precipitation, closely followed by mean annual air temperature, soil organic carbon, net primary productivity, pH, tree age, tree height, litter fall biomass, leaf area index, elevation and diameter at breast height.

Soil respiration can be measured with alkali traps or infrared gas analysers (IRGA), the latter being the current reference for CO_2 quantification (Davidson et al. 2002). Automatic and manual chamber systems that include IRGA analysers are commonly used to measure R_s and no significant differences between them have been found (Davidson et al., 2002; Irvine and Law, 2002; Savage and Davidson, 2003). However, these two types of chamber systems do differ in their cost and operational requirements. The use of automated chambers implies a higher equipment cost, allowing higher frequency of measurements at a lower operational cost. In contrast, manually-operated chamber systems are cheaper to buy and allow a higher spatial resolution, but with a higher operational cost. This implies that measurements with the latter type of chambers are usually done with a lower measurement frequency and will less likely include measurements during the night. Based on a review by Gomez-Casanovas et al. (2013), automated chambers are used to measure R_s in about 24% of the studies, while the rest used manual chambers. In the case of humid forests both approaches are used and, in the case of annual estimates using manual chambers, measurements are usually done only once per day and during daytime (Table 1). Regardless of the

type of sampling and which method is used to estimate the annual flux, little is known about how many measurements should be taken and at what time, in order to obtain more accurate estimates of daily and annual R_s.

Temperate forests present high shaded area compared to agricultural and ecosystems with sparser vegetation, such as pasture or shrublands, nevertheless, variations of $\pm25\%$ of the daily R_s flux has been reported for a temperate mixed hardwood forest, being the mid-morning measurements the best period to estimate daily mean fluxes (Davidson et al., 1998). However, errors in daily estimation of R_s can be generated if measurements are predominantly made during the warmest part of the day, thus introducing a bias in the estimation (Davidson et al., 2002).

Commonly researchers select times of the day during the morning, to get the estimates of daily R_s fluxes (Table 1). For example, Tang et al. (2006) suggested that measurements taken at 9:00 AM were representative of the daily mean flux in subtropical forests, however this value was calculated on the basis of 10 measurements made during the morning. This idea was tested by Qin and Yang (2013) using ecosystem respiration data which demonstrated that measurements taken at 9:00 AM were significantly higher compared with the daily mean. Similarly, Davidson et al. (1998) found that their measurements made between 9:00 AM and 12:00 PM adequately represented the average daily R_s flux in a temperate mixed hardwood forest, although this conclusion was derived from intensive measurements made on only two consecutive days. It is not clear why researchers prefer to measure in the morning (Table 1), but it may be because they are following published recommendations from other sites or it is related to the use of normal working hours.

Based on the estimation of seasonal or annual R_s, Savage et al., (2008) developed a protocol for data quality assurance in a mixed hardwood forest and determined that a sampling strategy with a bi-weekly frequency would be optimal. However, sampling was done only between 9:00 AM and 3:00 PM. Gomez-Casanovas et al. (2013) used high-frequency data to estimate the performance of different gap filling techniques to estimate annual R_s in experimental plots. As expected, they found that increasing the data gap fraction decreased the ability of all gap models to accurately predict R_s (above 15% decrease) and increased the variability of the prediction.

Using a high frequency sampling scheme (24 measurements per day) during one year in a temperate rainforest, we aimed to answer the still open questions of when and how many measurements per day and per year should be performed in order to adequately estimate R_s fluxes. Assessing performance of estimators based on their bias, precision and accuracy, as proposed by Walther and Moore (2005), our objectives were: (i) to assess the performance of estimating daily R_s fluxes based on different number of measurements per day, (ii) to compare the performance of estimating the annual R_s flux using linear interpolation or modelling based on different number of measurements per year, and (iii) to analyse the effect of including night-time measurements on the accuracy of the estimation of daily and annual R_s.

2 Methods

2.1 Study site

The study was carried out in a temperate rainforest at the Senda Darwin Biological Station (Carmona et al., 2010), a Long Term Socio-Ecological Research site located 15 km east of Ancud, in the Chiloé Island, Chile (41° 52' S, 73° 40' W)
5   (Figure 1). The dominant species are large emergent trees (up to 25 m) of *Drimys winteri*, *Podocarpus nubigena*, *Nothofagus nitida*, and *Saxegothaea conspicua*, while the understory species are seedlings and saplings of the dominant trees and some tree species of shrub habit, such as *Tepualia stipularis*. Tree trunks and fallen logs are covered by several bryophyte species, including mosses and liverworts. Soils are generally thin (<1 m), originated from Pleistocenic moraine fields and glacial outwash plains, with often poor drainage (Aravena et al., 2002). Soils are acidic (pH 3.9±0.4), with very low bulk density
10   (0.2±0.04 g cm^{-3}), high total C (39±9% dry weight) and low total N (1.3±0.2% dry weight) (Aravena et al., 2002). The climate is temperate with a strong oceanic influence. Meteorological records (1997-2008) at the Senda Darwin Station indicate an annual average temperature of 10°C, with a maximum average of 16°C in January and a minimum average of 5°C in July. Annual rainfall is 2.000-2.500 mm, with an average of 2.110 mm and a dry period during January-February. The year of the study (August 2013-July 2014) was wetter (2.383 mm) and cooler (9.4°C) than average, although the summer
15   months (December-February) were drier and hotter (Figure 2).

2.2 Automated measurements of R_s fluxes and environmental variables

The variability of forest conditions was preliminary assessed in terms of canopy cover and other stand related parameters. According to this, three soil respiration chambers were installed to cover the range of these variables, which were assessed in 3-m radius plots around each chamber. Table 2 shows the basic statistical parameters of the forest stand, soil and annual R_s
20   flux.

R_s fluxes were measured with an automated soil CO_2 flux system (model LI-8100, LI-COR, Lincoln, Nebraska, USA), connected to a multiplexer (model LI-8150, LI-COR) and three 20-cm diameter closed chambers (model LI-8100-104, LI-COR). The chambers were installed over a PVC collar buried into the soil, which stayed in place during the whole sampling period and were kept free of photosynthetically active material. Measurements were done for 2 minutes on each chamber
25   every hour, for one year, starting in August 8, 2013. Considering power supply problems that occurred on 6 days during the sampling period, the total data set was 25,776 records (24 measurements per day × 358 days × 3 chambers).

Soil temperature (T_s) was monitored at 5 cm depth, close to each chamber, using thermocouple probes (model TCAV, Campbell Scientific Inc. (CSI), Logan, UT, USA). Soil water content (Θ, m^3 m^{-3}) was measured at the same depth using water content reflectometers (model CS616, CSI) and corrected using field measurements of gravimetric water content and
30   soil bulk density. Data were collected with a datalogger (model CR3000, CSI) every 30 minutes.

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2.3 Generation of partial-data series for estimating daily R_s fluxes

For analysing the effects of making a different number of measurements per day and including or not night-time measurements on the performance of daily R_s flux estimations, we generated partial-data series. For this, we established four premises:

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1) The average from the three soil chambers represented the true forest R_s flux, in terms of its temporal variation.

2) The average of the 24 measurements made in one day (00:00 to 23:00) was considered to be the best estimate of daily R_s flux,

3) Daily sampling was made at frequencies of 1, 2, 4 or 6 measurements per day, which were averaged to estimate the partial-data daily R_s flux.

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4) Two sampling types were defined, considering all the measurements made during one day (*day-night*), or considering measurements made only during daytime (07:00 to 19:00, *day*).

The partial-data series were then generated by randomly selecting measurements. This process was different for the two sampling types defined in 4):

a. For the day-night sampling, after randomly selecting one day (out of the 358 possibilities), the initial time

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of sampling was also randomly selected (out of the 24 possibilities); the other time(s) of measurement was selected equidistantly from this initial value, maximizing the time distance between samplings to fit the number of measurements in one day. In the case of 1 measurement per day, the time of measurement was the same as the initial time of sampling.

b. For the day sampling, to maintain the number of measurements per day and to make them as equidistant as

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possible, the time of measurements were randomly selected from windows of time, as shown in Figure 3.

2.4 Generation of partial-data series for estimating the annual R_s flux

We defined different frequencies of sampling assuming that the most common sampling schemes are seasonal (summer, autumn, winter and spring), bimonthly, monthly, biweekly or weekly. These frequencies implied 4, 6, 12, 26 and 52

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measurements per year, respectively, which represented our partial-data series. The best estimate of the annual R_s flux was calculated considering all available data. Because the error in estimating the annual R_s flux is not independent of the number of measurements during one day, we combined the daily and annual frequencies of sampling. The day and day-night sampling types were also considered for the estimation of annual R_s flux using partial sampling. The days of measurements were determined by selecting a random initial day of measurement and then adding (or subtracting) the maximum possible

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time distance to fit the number of measurements within a year. In addition, a buffer range of days was added around the days originally selected to simulate the fact that, in most field studies, the real day of measurement is not exactly the planned one.

This buffer range varied with the frequency of measurement, being ± 16, 8, 4, 2 and 1 day, for the frequencies of 4, 6, 12, 26 and 52 measurements per year, respectively.

Once the selection of daily and annual measurements was done, we used two different approaches to estimate the annual R_s flux. The first approach was linearly interpolating the daily fluxes, while the second one was modelling based on environmental variables. For selecting the model, we used all the available data and found that Van 't Hoff equation based on T_s was the most appropriate model for our data set ($R_s = 0.744$ $e^{(0.13 * Ts)}$; $R^2=0.89$) : although soil water content did show a significant negative linear relation with R_s, adding this variable to the model did not improve the adjusted coefficient of determination, so the simplest model (based only on T_s) was preferred.

2.5 Performance of estimations of daily and annual R_s fluxes using different sampling frequencies

For estimating the performance of the estimations of R_s, we used unscaled measures of bias, precision and accuracy, according to Walther and Moore (2005). Bias is defined as the deviation of measurements from the mean, which is usually due to faulty measuring devices or procedures. Bias therefore leads to either underestimation or overestimation of the true value. Precision is the statistical variability of an estimation procedure and is considered to be independent of the true value. Finally, accuracy defines the overall performance of an estimator and is the combination of bias and precision.

All three parameters were calculated for each sampling frequency based on 10,000 partial-data series, which were generated as described in sections 2.3 and 2.4. The Bias of the daily estimation for each frequency of sampling was calculated as:

$$Bias = \sum_{\substack{1 \le i \le m \\ 1 < j < n}} Rs_{ij} - Rsb_{ij} \, , \tag{1}$$

where Rs_{ij} is the partial-data estimation of daily Rs flux and Rsb_{ij} is the best estimate of the daily R_s flux, for sample i (m=10,000 iterations) and day j (n=358).

The precision was estimated as the standard deviation (SD) of the partial-data series estimations of the daily flux, using the values selected in Equation 1:

$$SD = \left(\frac{\sum_{\substack{1 \le i \le m \\ 1 < j < n}} (Rs_{ij} - \overline{Rsb_{ij}})^2}{m} \right)^{1/2} \tag{2}$$

Finally, the accuracy of the estimation of daily R_s for each frequency of sampling was estimated as the root mean square error (RMSE), which was calculated as:

$$RMSE = \left(\frac{\sum_{\substack{1 \le i \le m \\ 1 < j < n}} (Rs_{ij} - Rsb_{ij})^2}{m} \right)^{1/2} \tag{3}$$

All hourly values and days had the same chance of being selected. For estimating the performance of estimations of annual R_s flux, the same three statistical parameters (Equations 1-3) were used, considering as best estimate the annual R_s flux calculated using all available data. All the statistical analyses were done using the software R, version 3.1.2 (R Core Team, 2014).

3 Results and Discussion

3.1. Daily and annual R_s fluxes and environmental variables

The daily R_s showed in cold months (June-September) values around 7 g CO_2 m^{-2} day^{-1} and up to 20 g CO_2 m^{-2} day^{-1} in summer (mid-January) (Figure 4-A). There was low variability of daily R_s values between chambers, shown by the low coefficients of variation of each season (i.e., the average of daily variations between chambers): winter 15.9%, spring 18.3%, summer 18.9%, fall 12.6% (Figure 4-A). Although we used only three soil chambers, we think these represented correctly the temporal variation of R_s fluxes in the forest. For recommendations on the number of samples to represent the spatial variation, see Davidson et al. (2000) and other related publications.

The same pattern was observed for T_s, which moved from values around 6 °C during the winter, up to a maximum of 16 °C in the middle of the summer (Figure 4-B). The opposite pattern was observed in Θ, where the highest and very stable values were observed during the winter months around 0.55 and decreased during the summer to around 0.4 (Figure 4-C). A greater variability between chambers was observed in Θ compared to T_s (Figures 4-B and 4-C).

The difference in R_s between winter and summer periods may have been increased by the fact that during the study year, winter was wetter and cooler than average, while the summer months were drier and hotter (Figure 2). This situation matches the prediction of climate change in this area for the period 2071-2100 reported by Fuenzalida et al., (2007). If this dryer summer conditions continue, a reduction is expected for evapotranspiration (15%) and aboveground biomass (27%) in this ecosystem type (Gutiérrez et al., 2014).

3.2. Effects of sampling frequency and nighttime measurements on daily estimations of R_s

In the scenario where only daytime measurements were considered, the bias was always positive, around 0.35 g CO_2 m^{-2} day^{-1}, meaning an overestimation of daily R_s fluxes (Figure 5-A), which coincides with the suggestions of Davidson et al. (2002) and Qin and Yi (2013). Increasing the number of measurements in this scenario from 1 to 6 per day decreased the bias only slightly. Including night-time measurements decreased the bias for estimating daily R_s fluxes to nearly zero (Figure 5-A), with little difference observed when making 2, 4 or 6 measurements per day.

The precision (SD) of the daytime scenario was around 4.2 g CO_2 m^{-2} day^{-1}, showing only a small decrease when adding more measurements during the day (Figure 5-B). Adding measurements during the night decreased the SD only 5% to a value of 3.99 g CO_2 m^{-2} day^{-1}.

The RMSE for only daytime measurements showed an important decrease when comparing the frequencies of 1 and 2 measurements per day (1.45 and 0.82 g CO_2 m^{-2} day^{-1}, respectively) (Figure 5-C), while still decreasing but less with 4 and 6 measurements per day. Adding nighttime measurements decreased the RMSE considerably, especially for sampling frequencies ≥ 2, which had a mean RMSE of 0.39 g CO_2 m^{-2} day^{-1}.

The precision of daily measures of R_s was one order of magnitude higher than the bias, when comparing only daytime measurements, difference that was much larger when considering day and night measurements (Figures 5-A and B). According to our results, the best option was to measure twice during the day, even if both measurements were restricted to daytime, because this frequency yields an RMSE <10% of the mean daily value (Figure 5-C). If the accuracy threshold was to be set at 5%, the minimum frequency of sampling required was 4 times per day, including night-time measurements.

3.3 Effect of frequency of sampling and inclusion of night-time measurements on annual estimations of R_s

Figure 6 shows the statistical parameters for the annual estimations of R_s using both linear interpolation and non-linear modelling approaches. This figure does not include the results of 6 measurements per day because they were almost identical to measuring 4 times per day. All the results are shown in Supplementary material Table S1. In the scenario of only daytime measurements, the bias was always positive around 0.12 kg CO_2 m^{-2} $year^{-1}$ for all frequencies of daily sampling (Figure 6-A). Adding nighttime measurements made the bias negative, but closer to zero (Figure 6-B). Only in the latter case using modelling, instead of interpolation, performed better, i.e. showed a bias closer to zero.

In summary, regardless of the annual frequency of sampling, making measurements only during daytime represented a positive bias (overestimation) of the annual R_s flux. Sampling also during nighttime practically eliminated the bias. However, this does not mean that error does not exist, but only that this error occurs equally above and below the mean. The error (defined as the observed – modeled values) decreases greatly when moving from sampling 4 to 6 per year (Supplementary material Figure S1).

The SD of only daytime measurements moved from a maximum around 0.52 kg CO_2 m^{-2} y^{-1} for the frequency of sampling 4 days per year and measuring once per day, to a minimum 0.07 kg CO_2 m^{-2} y^{-1} for the frequency of 52 per year and 4 per day (Figure 6-C). There was an improvement (lower SD) when adding nighttime measurements (Figure 6-D) and more so when using a modelling approach to estimate the annual R_s flux. This latter improvement was more noticeable when sampling 2 or 4 times per day and 12 times per year.

Because the parameter we used to represent the precision of the annual estimation (SD) accumulates the difference between observed and modeled values, the magnitude of the error associated to precision was much larger than the bias, making the value of precision almost identical to the accuracy parameter (Figures 6-C and -D compared to 6-E and -F).

The RMSE of the daytime scenario showed a decreasing trend when increasing the frequency of sampling from 4 to 6, 12, 26 and 52 days per year, with mean values of 0.42, 0.34, 0.26, 0.22 and 0.20 kg CO_2 m^{-2} y^{-1}, respectively (Figures 6-E and -F). Within each annual frequency of sampling, there was a considerable decrease in the RMSE when comparing the frequency 1 per day with the frequencies 2 and 4 measurements per day. In the day-night sampling (Figure 6-F), the RMSE decreased between 0.01 - 0.11 kg CO_2 m^{-2} y^{-1} compared to the corresponding partial-data series of daytime sampling. The non-linear modelling approach performed better than linear interpolation only when sampling was performed day-night

(Figure 6-F). When sampling only during the day (Figure 6-E), the modelling approach was clearly better only when sampling 2 or 4 times per day and 12 times per year.

If sampling was done only once a day, sampling once a month was the minimum frequency required for obtaining accurate estimates of annual R_s (RMSE <10% of the annual flux) (Figures 6-E and -F). When establishing a threshold of accuracy of
5   5%, for sampling only during daytime, a minimum of 2 measurements per day, and biweekly field campaigns were required (Figure 6-E). For the latter accuracy threshold, if nighttime measurements were included, sampling twice a day was also required but only at a monthly interval (Figure 6-F). This example highlights the importance of performing nighttime measurements.

The effect of sampling more times per day on the error of annual R_s estimation was more dramatic when moving from 1 to 2
10 measurements per day, less so when moving from 2 to 4 and almost negligible when moving from 4 to 6 (Figures 6-E and -F). On the contrary, increasing the number of field campaigns (days sampled per year) showed a continuous and almost linear decrease when moving from 4 to 52 days per year (Figures 6-E and -F).

3.4 Other sources of error and means of improving the accuracy of R_s estimations

Table 1 shows that there is great variability in both daily and annual frequency of sampling in studies that measured R_s in
15 humid forests. Most studies that used manual chambers sampled only once per day, only during daytime and between 6 and 48 times per year. According to our results, only the estimations from studies that sampled annually ≥12 times would yield an RMSE <10% of the annual flux.

We tested both linear interpolation and modelling based on soil temperature as gap filling approaches, expecting that the latter would yield lower error in the annual R_s flux, as suggested by several authors (Davidson et al., 1998; Raich and
20 Schlesinger, 1992; Wang et al., 2006). However, the modelling approach represented a clear decrease in error only when sampling 2 or 4 measurements per day and once a month, which in our study represented an intermediate number of total measurements per year. This makes sense, given that fitting a non-linear model requires a minimum number of data, over which modelling performs almost equal than interpolation. Gomez-Casanovas (2013) compared nine different gap-filling methods and concluded that the linear interpolation method was the second best-performing method, while the method based
25 on T_s was among the most poorly-performing methods. Here we found that modelling based on T_s was a better method, particularly in reducing the bias, but not as expected.

Unfortunately, we cannot compare our results with the studies summarized in Table 1, because no information is given about the level of accuracy of the daily or annual estimations of R_s flux. Because our study site is very close to the ocean, daily and annual climatic variability are low. We expect that obtaining good performing estimates of daily and annual R_s fluxes
30 under more variable environmental conditions would require more frequent sampling. This is expected not only because of the larger diurnal and annual oscillations of soil temperature and humidity, but also because of the higher variability in biological activity of trees during the year.

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Finally, we agree with Gomez-Casanovas et al. (2013), in relation to the need of improving and standardizing the techniques to estimate the annual R_s for understanding its role in the global C cycle. According to our results, part of this standardization process should not only include the gap-filling approach, but also the frequency of daily and annual sampling. Accomplishing this will require studies similar to ours under different environmental conditions.

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4 Conclusions

According to our observations in a temperate rain forest site, if the research question seeks accurate daily estimations of R_s, sampling ≥ 2 per day would be necessary to obtain an accuracy that represents an RMSE <10%. Adding nighttime measurements improved the accuracy and precision slightly and, most importantly, decreased the bias, which was always

10 positive when sampling only during daytime.

In general, accuracy of most combinations of daily and annual sampling frequencies used for modelling annual R_s achieved high accuracy (RMSE<10%). The reduction in RMSE was highest when increasing measurements from 4 to 6 per year, but was still relevant when further increasing annual measurement frequency. We therefore recommend increasing annual rather than daily measurement frequency and including a minimum of one daytime and one night-time measurement. Actually, in

15 the case of establishing a high accuracy threshold (RMSE<5%), making one of the two measurements during the night in one day, decreased the number of field campaigns per year from 26 to 12.

The decrease in error when using modelling instead of linear interpolation for estimating R_s annual flux was evident only for intermediate sampling frequency levels which, in our case, was represented by doing 2 or 4 measurements per day and field campaigns once a month.

20 As a general measure for reducing the errors originated from partial sampling of R_s during one day and during the whole year, we recommend making an intensive sampling (including nighttime measurements) at the beginning of the study. This should allow determining the best time(s) and the minimum frequency for sampling. We expect that this process may be more critical where environmental conditions are more variable compared to the conditions in our study site.

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Table 1. Sampling schemes of studies where annual R_s was estimated in humid forests.

Forest type (country)	Period	Sampling time	Daily freq.	Annual freq.	Type of sampling	Method for estimating annual flux (variables used)***	Reference
North temperate forests (USA)	32	-	1	9**	A	Linear interpolation	Fisk et al. (2004)
Temperate mixed-hardwood forest (Korea)	8	-	1	9	M	Non-linear model (T_s)	Kang et al. (2003)
Temperate beech forest (France)	18	7:30 - 16:00	1	12 - 24	M	Non-linear model (T_s, Θ)	Ngao et al. (2012)
Coniferous forest (China)	12	10:00 - 12:00	1	24	M	Non-linear model (T_s)	Xu et al. (2015)
Mixed hardwood old-growth forest (USA)	12	9:00 - 15:00	1	25	M	Linear interpolation	Savage et al. (2008)
Mixed hardwood forest (USA)	12	9:00 - 13:00	1*	28	M	Linear interpolation and Non-linear model (T_s)	Davidson et al. (1998)
Mixed and broadleaved forest (China)	12	09:00	1	48	M	Linear interpolation	Tang et al. (2006)
Temperate mixed forest (Belgium)	12	-	2	12	M	Non-linear model (T_s, Θ)	Curiel Yuste et al. (2005)
Subtropical forest (China)	12	9:00 - 12:00	4	48	M	Non-linear model (T_s)	Yan et al. (2006)
Subtropical forest (China)	12	0:00 - 23:00	24	12	A	Non-linear model (T_s)	Yan et al. (2006)
Cool-temperate deciduous forest (Japan)	36	0:00 - 23:00	24-48	12 - 24	A	BGC-model (T_s, T_a, Pp, VPD)	Kondo et al. (2015)
Cool-temperate deciduous forest (Japan)	48	0:00 - 23:00	24-48	12 - 24	A	Non-linear model (T_s)	Mo et al. (2005)
Mixed hardwood old-growth forest (USA)	12	0:00 - 23:00	48	180	A	Linear interpolation	Savage et al. (2008)

* Assumed to be 1 because no information was reported. **Winter months were estimated from a different study.

*** Abbreviations are: T_s, soil temperature; Θ, soil water content; T_a, air temperature; Pp, precipitation; VPD, vapor pressure deficit.

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Table 2. Characteristics of the forest stand, soil (30 cm) and annual R_s flux (n=3).

Attribute	Mean	SE	Min	Max
Soil bulk density (g cm^{-3})	0.49	0.2	0.16	0.85
Soil carbon content (%)	40.16	1.65	19.24	54.10
Total litter biomass (Mg ha^{-1})	18.3	2.8	6.4	28.6
Litter biomass Oi layer (Mg ha^{-1})	6.4	0.9	1.9	10.9
Litter biomass Oe layer (Mg ha^{-1})	11.9	2.2	3.2	18.8
Total root mass (Mg ha^{-1})	23.6	6.5	7.0	70.1
Coarse root mass (Mg ha^{-1})	16.4	5.8	3.5	58.9
Fine root mass (Mg ha^{-1})	7.1	0.9	3.3	11.1
Canopy cover (%)	84.3	7.35	69.7	92.95
DBH (cm)	11.13	9.44	5	65
Density (trees ha^{-1})	1521	623	354	2475
Total basal area (m^2 ha^{-1})	44.68	41.69	1.13	128.04
Annual R_s flux (kg CO_2 m^{-2} year^{-1})*	4.15	0.31	3.81	4.76

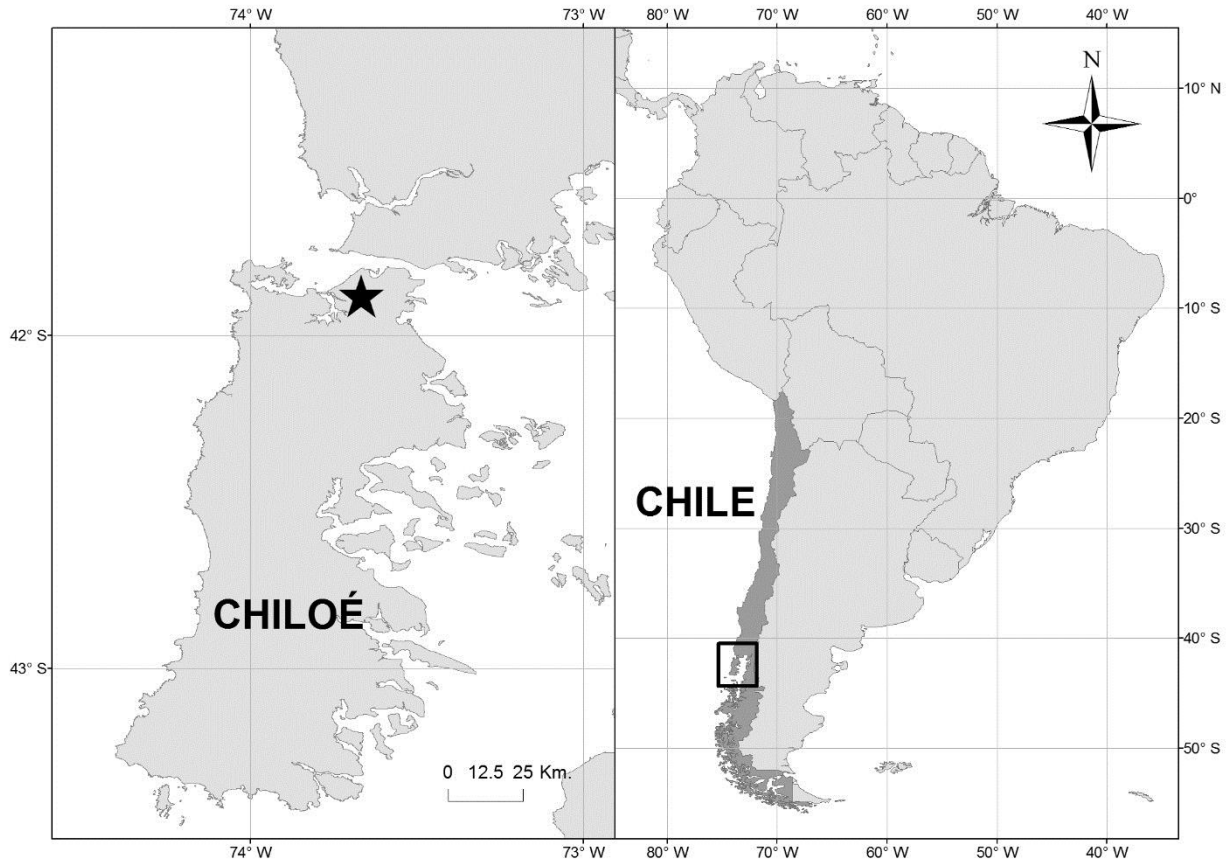* The true R_s flux was estimated using the whole-data series.

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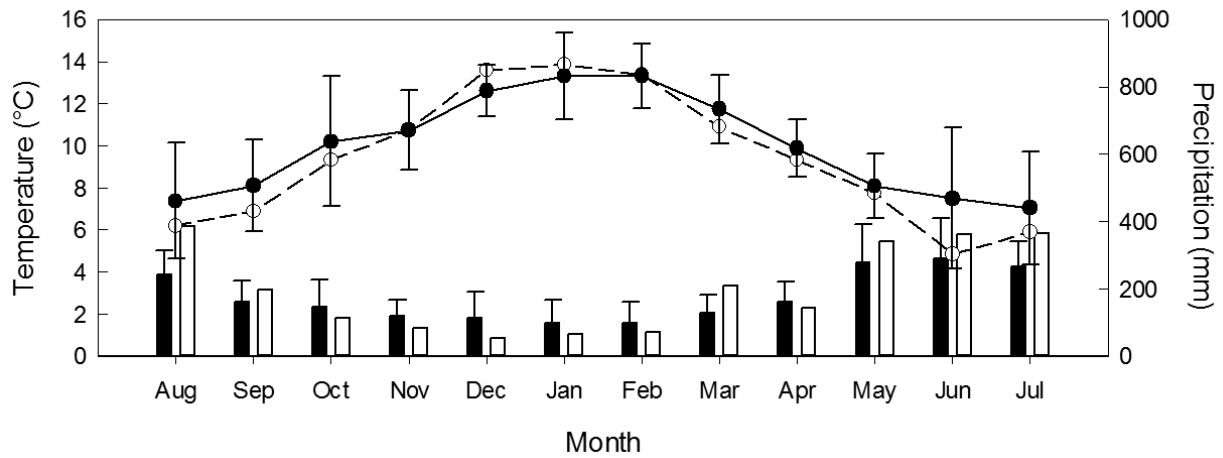**Figure 1: Location of the Senda Darwin Biological Station (marked with a star) at the Chiloé island.**

Figure 2: Mean long-term (1999–2012) (black) and August 2013-August 2014 (white) monthly precipitation (bars) and air temperature (circles).
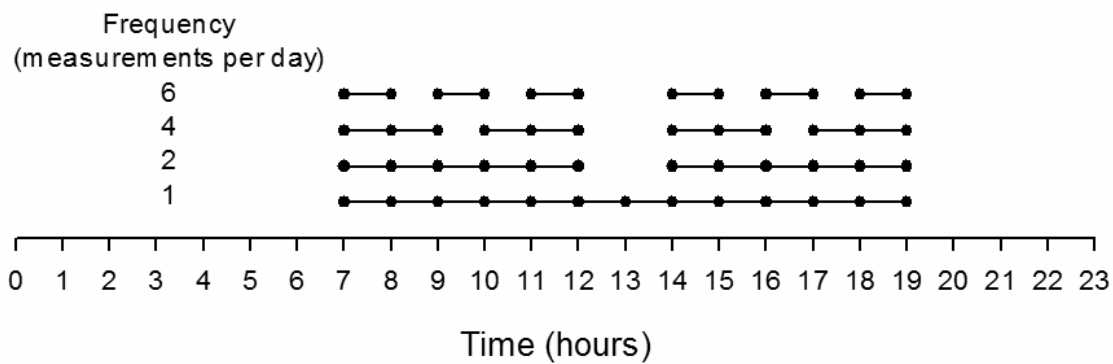
Figure 3. Windows of time for randomly selecting measurements of different sampling frequencies, for the only day-time sampling. The lines represent the windows of time and the dots represent the exact time of measurements.
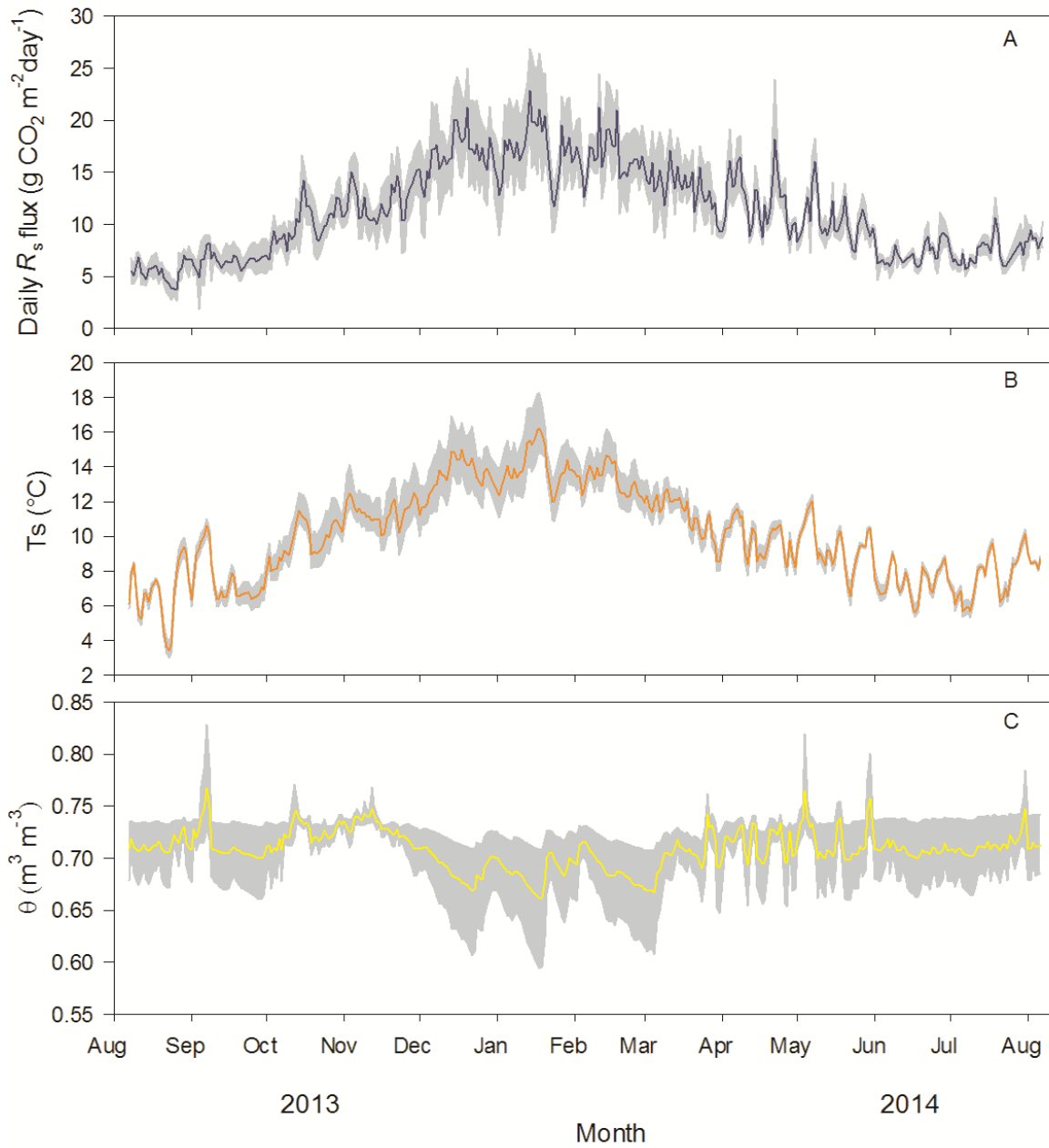
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Figure 4. Daily mean R_s (A), soil temperature (B) and soil water content (C). Shaded area represents the minimum and maximum range of three sampling points.
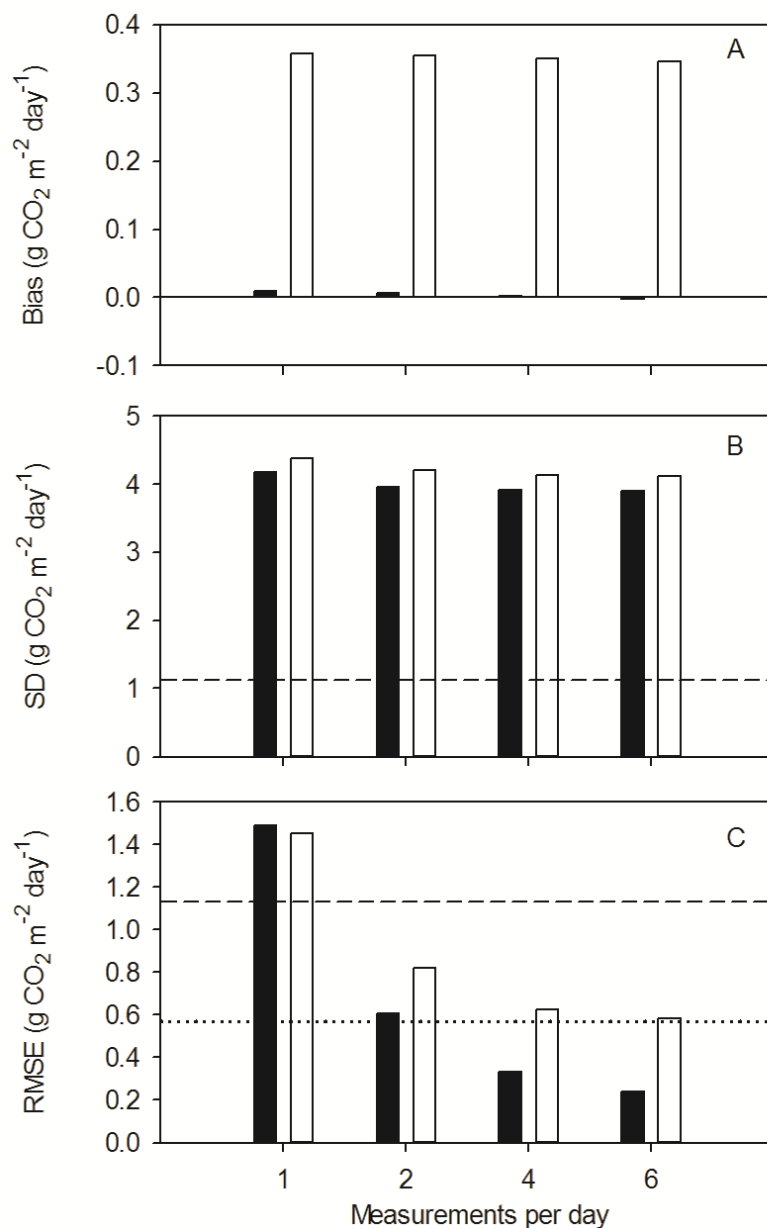
Figure 5. Statistics of the estimation of daily R_s flux from partial-data series, considering day and night measurements (dark bars) and only daytime measurements (light bars) ($n=10,000$). Statistical parameters are bias (A), standard deviation (B) and root mean square error RMSE (C). The reference lines in panels B and C represent 10% (dashed) and 5% (dotted) of the mean daily R_s flux (11.38 g CO_2 m^{-2} day^{-1}).
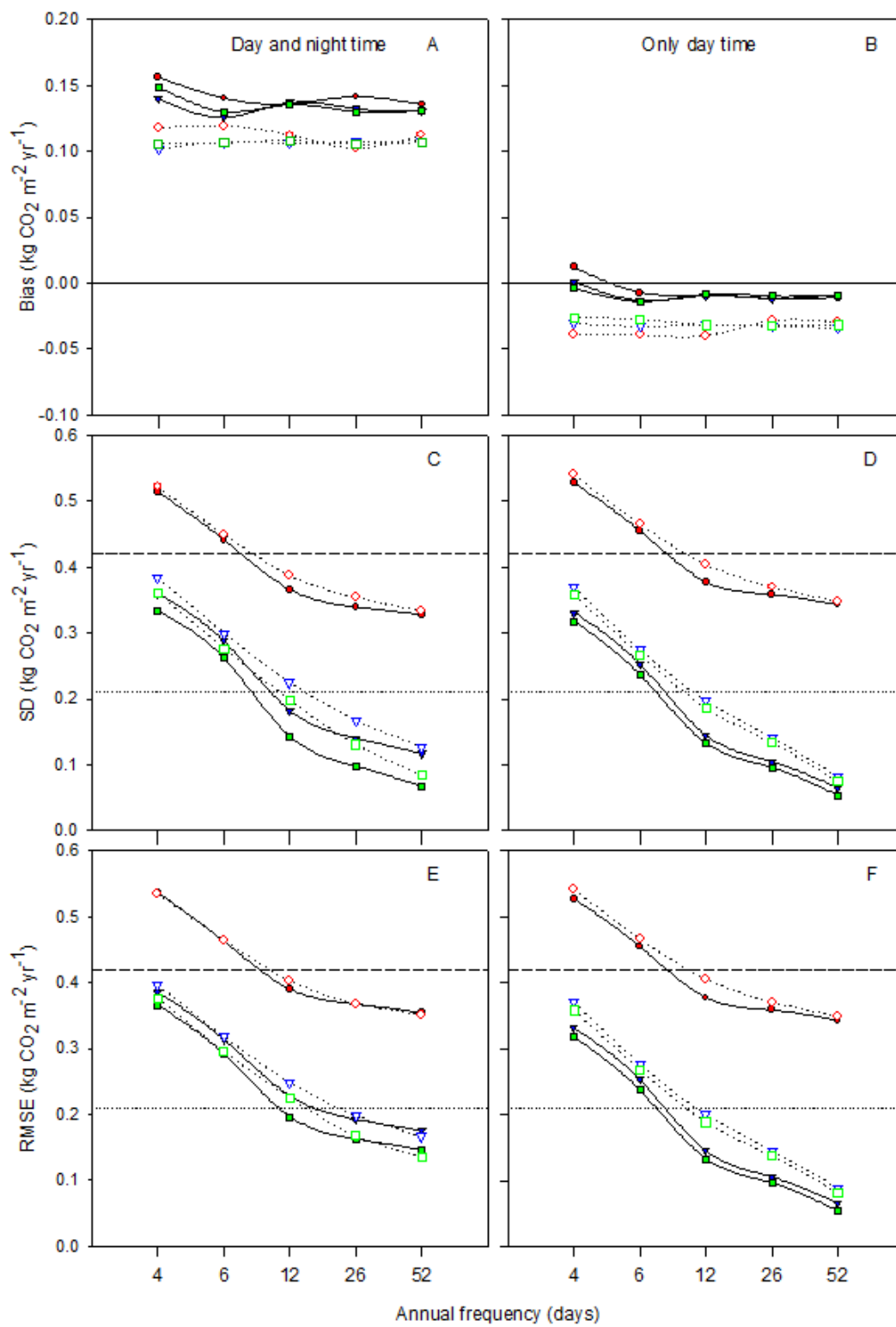
Figure 6. Bias (A, B), SD (C, D) and RMSE (E, F) of partial-data series, related to both daily and annual frequencies of sampling, considering only daytime measurements (left), or day-night measurements (right). Symbols represent the daily frequency of sampling: red circle, 1; blue triangle, 2; green square, 4. Frequency of 6 measurements per day was not included to make the graph clearer, because the error values did not differ much from the ones for 4 measurements per day. The approach to estimate the annual flux are differentiated as follows: blank symbol and pointed line are linear interpolations; solid symbol and solid line are non-linear modelling. The reference lines in C, D, E and F panels represent 10% (dashed) and 5% (dotted) of the annual R_s flux (4.15 kg CO_2 m^{-2} y^{-1}).

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