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Authors’ response to reviewers’ comments

General comments

We thank all of our reviewers and our pleased to see that our manuscript has been positively received. We have already addressed short comments SC1-SC3 and here we respond to the outstanding short comment SC4 and the reviewer comments RC1-RC3.

Our reviewers provided some insightful criticisms and several relevant additional sources which we have incorporated into the revised manuscript. Whilst it was not possible to implement all the suggested changes, principally due to contrasting opinions of the reviewers, we have endeavoured to implement as many as possible in a logical manner. Our responses (in red) to specific reviewer comments are found below.

Reviewer 1 (RC1) J.W. Atkins (Referee)

Specific comments

Pg 1, line 26 need an “of” between “amounts” and “carbon”

Response: amended.

Pg 2, line 4 – this sentence is worded awkwardly and could be focused more. Monitoring would imply fixed-chambers, but many studies employ portable chambers some of which require in-situ collars and some that don’t.

Response: altered ‘monitored’ to ‘employed for sampling’.

Pg 2, line 13 – “these studies have focussed on a single vegetation type or land use thus do not resolve . . .” Focused has an “s” too many and you need a conjunction between “use” and “thus.”

Response: We recognise that US English may prefer ‘focused’ but according to the journal’s guidelines, we have consistently used UK English our manuscript, hence ‘focussed’. Addition of comma instead of conjunction.

Pg 2, lines 10-20 – There is some work from Diego Riveros-Iregui that would be a valuable contribution here about diurnal hysteresis if not in this section to set the scene, perhaps later: Riveros-Iregui, D. A., Emanuel, R. E., Muth, D. J., McGlynn, B. L., Epstein, H. E., Welsch, D. L., ... & Wraith, J. M. (2007). Diurnal hysteresis between soil CO2 and soil temperature is controlled by soil water content. Geophysical Research Letters, 34(17).

And this paper may also be useful:

Ruehr, N. K., Knohl, A., & Buchmann, N. (2010). Environmental variables controlling soil respiration on diurnal, seasonal and annual time-scales in a mixed mountain forest in Switzerland. Biogeochemistry, 98(1-3), 153-170.

Response: We appreciate being pointed to these relevant studies and refer to them later in the text (p4 L6).

Pg. 3, line 30 – just a note on units grams per hour or micromoles per second are typically more common in the literature.

Response: We recognise that units of mass can cause confusion and have therefore made a clarification: all fluxes are now explicitly expressed in (g CO_2 mg m-^{2} h^{-1})

Pg. 4, lines 1-3 – I don't completely understand what you are saying with this phrase,“ . . . and daily means at 09.00 and ca. 20.00 for all three months in barley” could you clarify that? Are you saying that is when fluxes approximate daily means? And a really, really minor point, but I think “greater” works better to describe fluxes than “higher” because you are talking about a magnitude, an accumulating sum of sorts.

Response: Reworded sentence for clarity “For all three months in barley, maximum R_s was seen between 12:00-15:00, minimum around 05:00 and daily means at 09.00 and *ca*. 20.00.”. Amended ‘higher’ to ‘greater’.

Pg. 3, lines 16-20 – Love it. That is a great point and I am enthused to see this work on experimental design and sampling! That is a good highlight to show that difference and make that point about missing differences between the systems.

Response: We are glad that this is appreciated!

Pg. 4. – It would be helpful if you showed your soil moisture data or described it in some way and provide analysis of how that is working with temperature or in isolation to control fluxes. That interaction can be important. There are various ways to look at the interaction of temperature and moisture such as an ANCOVA or even looking at some log regression detrending. Inclusion of an ANCOVA would likely address this and be of minimal additional work.

Response: We reference multiple regression analyses which show that soil moisture is not important on the diurnal scale “Inclusion of soil moisture in a multiple regression did not improve the model, indicating that soil moisture does not affect R_s on the diurnal scale”.

Fig. 1 – there is a bit of an over-plotting issue with the data that could be addressed by perhaps widening the plot or decreasing the marker size.

Response: The figure has been amended to include a separate panel for each crop for clarity.

Fig. 2 – Great plot in general, but I think that changing the scale on the y-axes, though I understand visually why it was done, is not a good practice. Normalizing those axes would also better show monthly differences as you can see in the soil temp. plots at the bottom.

Response: We have amended the figure so all the y axes are consistent.

Reviewer 2 (RC2) Anonymous referee #1

Specific comments:

P. 1, l. 8-10: I don't follow the logic of the sentence. The first half refers to a mode of measurement, based on “convenience” of working in daylight hours, the second invokes an assumption that temperature is a dominant control of soil CO_2 efflux. Why this conflation? I assume you want to set up the issue of contrasting diurnal maxima periods, but this is not at all clear in the way it is phrased.

Response: Amended the sentence, replaced “with” with “convenience which is justified by”. Our aim here is not to conflate two issues but illustrate how a tacit assumption that diurnal variation in R_s will be controlled by soil temperature (as we show through the literature cited), and the further assumption that this is consistent at a single location (as shown in our data), has encouraged a sampling convention that synchronous measurements will facilitate valid comparisons to be made, which might not be the case.

P. 1, l. 12: The statement that R_s in Miscanthus peaked in the night is not true. For May and June, Miscanthus "peaks" during the 9:00 – 16:00 window. Diurnal variations in July are subtle (+/- 10%), and interpretation should take account of this magnitude.

Response: This sentence has been re-worded: "whereas in *Miscanthus* after an initial early evening decline, R_s increased above the daily average during the night and in July maximum daily rates of R_s were seen at 22.00".

P. 2, l. 3-7: Here is the same conflation of measurement mode and temperature control. The two concepts are not logically linked here – the single measurement is not a consequence of temperature being widely held as a dominant control on respiration, as the sentence suggests.

Response: An amendment has been made to provide further clarity and to avoid conflation: "and if combined with an assumption that soil temperature will be consistent across a single site, a logical expectation might be that the diurnal variation in R_s will also be consistent at that site".

p. 3, l. 30: As you chose to express fluxes on a mass basis, please specify whether these are grams of carbon, or grams of CO_2. Using molar units would avoid any confusion.

Response: For clarity we have amended flux units to mg CO_2.

P. 4, l. 12: "fully"???

Response: Now "as much as".

P. 4, l. 17: Which protocol do you refer to here?

Response: Re-worded the sentence for clarity "if a protocol which used the same sampling hour were used over several months".

P. 4, l. 30-32: Picking-and-choosing your data points so they fit the narrative is not appropriate. The temperature response for both data series have to be balanced, and you should show all hourly data for Miscanthus in Fig. 4. Or, as you are interested in a temperature regression across all months, I'm not sure that hourly data are meaningful to show in any case. It's a shame that you don't have temperature data for June in Miscanthus, but for a seasonal temperature response (which is what is sown by regression lines in Fig. 4), you can use monthly average Rs and soil temperature measured in barley as an approximation. From Fig. 2, this would place fluxes of around 300 mg CO2 m-2 h-1 near 12 deg C – what does that do to your curve? Regarding your regression functions – is an apparent saturation curve for the temperature response meaningful for Miscanthus? Finally, there seems to be a mismatch between short-term temperature response (e.g. June in barley, where diurnal flux response to temperature change is very sluggish) vs. seasonal response – this may be worth commenting on.

Response: We acknowledge that our analysis might be perceived as subjective, therefore we show a balanced comparison of all hours for both crops and for the hours of the measurement window in an amended Fig. 4.

P. 5, l. 5: delete "however" (not needed as you start the sentence with "although")

Response: Deleted

P. 4, l. 32 – p. 5, l. 2: I don't completely follow this analysis. Why do you suppose that the relationship between solar radiation and soil CO2 flux is linked to the "typical measuring window"? It seems an entirely arbitrary separation of daytime/nighttime of your data set. What I can see in these graphs is that by introducing a time shift between two essentially sinusoidal curves, you can create an apparent correlation. . The same analysis would work for soil temperature with a time lag, but I obviously see what you're getting at with the lag analysis. An analysis of regression between instantaneous flux and preceding photosynthesis (or radiation used as a proxy) would be more meaningful. If what you try to show is the case, then the deviation from the mean in CO2 flux should be greater during nights following days with high radiation, and less following days of low photosynthesis (i.e. low radiation).

Response: The regression of R_s and time-lagged solar radiation is included in Fig. 5.

Figure 1: Placing both data series on top of each other is not helpful. Please split into separate panels. What happened around the 20th July in the barley field? It seems strange that fluxes should suddenly fall dramatically and then remain constant for days (with only little diurnal variation visible), to then jump back. Any hints in the meteorological data or management (harvest)? What is the impact on your diurnal calculations?

Response: Fig. 1 amended to show two panels. Soil moisture dropped below 0.16 m^3 m^{-3} for the only period of the study. This is now referenced (P5 L11): "however, after two weeks without rain, soil moisture dropped to a low of 0.16 m3 m-3 for a short period (19th – 22nd July) in the arable crop, during which time Rs dropped considerably. When heavy rainfall elevated soil moisture rates of Rs increased again which would suggest there is a threshold above which soil moisture is not limiting, an effect similar to that described by Xu and Qi (2001)".

Figure 3: I'm not sure that this graph provides much new information It should be the same as Fig. 2, only that average fluxes per hour and month are multiplied by the number of measurement days, or not? Dynamics should hence be identical.

Response: Whilst we acknowledge the reviewer's observation that the hourly dynamics of cumulative flux are the same as the diurnal variation in R_s values, we feel it is appropriate and useful to show the additional information of the cumulative flux when all hours are integrated, *viz.* the final columns of Fig. 3.

Reviewer 3 (RC 3) Anonymous referee #4

p1L14: "coincided with levels" - unclear, reword (see also comment on p5L32).

Response: This sentence has been reworded "Since the time of the daily mean R_s in *Miscanthus* occurred when R_s in the barley was 40% greater than the daily mean".

2.1 This section in general: How often and for how long were the chambers closed?

Response: We have moved a sentence from section 2.2 to 2.1 (P2 L32) for clarity: "The chambers were programmed to close for two minutes during measurement, with a 30 second 'dead band' to allow for mixing of the headspace in a continuous cycle between chambers".

p2L23: ...and *an* infrared gas analyser? p2L24: specify: was it 2 multiplexers (one per ecosystem?) p2L28: inserted 2 cm: It is not mentioned which collar height was chosen (Li-Cor's standard?) and/or how high they protruded above the soil surface. In general, an insertion depth of 2 cm is rather low (possible lateral diffusion in coarse soils) and the resulting large height above the surface should be avoided because of its altering effect on insolation, precipitation and wind (probably not so much an issue once the plant canopy is closed).

Response: Additional clause included (P2 L28): "with one IRGA and one multiplexer deployed in each crop". Whilst we acknowledge that there are effects of collar height, we ensured that these effects would be consistent between the two crops and agree with the reviewer that this was less of an issue since we were measuring under a canopy. We therefore prioritised concerns regarding cutting fine roots over the effects highlighted by the reviewer, and have included an additional reference (Heinemeyer et al.) to explain our reasoning- see methods section (P2 L31): "chambers were seated over PVC collars (diameter 20 cm, height 10 cm) which were inserted ca. 2 cm into the soil in order to minimise the effect of cutting fine roots (Heinemeyer et al., 2011)".

p2L30: Give more details on sensor installation (vertical or through a trench, resulting depth averaging). Note that to gain confidence in the later discussion on (partly lagged) responses to temperature and solar radiation, the temperature would ideally have been measured in several depths.

Response: Additional information "using vertically-installed sensors".

2.2 p3L6: Licor software and manual sounds a bit odd, maybe "manufacturer"?

Response: Re-worded: "using the manufacturer's software (see manufacturer's manual https://www.licor.com/documents/jtpq4vg358reu4c8r4id.pdf)".

p3L14:duplicate dot after 80%

Response: Deleted

p3L17-20: Try to secure the reproducability of the statistical methods not so much (or at least not only) by telling which option of the applied software was chosen, but rather by referring to the name of the test, to literature if necessary, etc., e.g. which test for normality? The result on normality does not seem to be mentioned in the results section (if I didn't overlook it). Note that for soil respiration in general it wouldn't be surprising if it was lognormal rather than normal, where necessary some authors work with log-transformed values.

Response: A clarification of statistical approach has been added to the methods with an appropriate citation (P3 L20): "The cumulative fluxes for the whole period were tested for normality using a Kolmogorov-Smirnov (K-S) test, but due to the size of the dataset this approach was unsuitable for the cumulative fluxes for sampling hour and instead limits of kurtosis and skewness of ± 2 were used as acceptable deviation from a normal distribution (Field, 2013). Differences in the whole-period cumulative flux were tested using one-way analysis of variance; the effect of crop, sampling hour and month were tested using a mixed-effects model accounting for the repeated estimated totals from each chamber for each month (PROC MIXED in SAS, using the 'repeated' statement and an autoregressive covariance structure)."

P4 L17: The results of tests for normality are reported: "The data did not significantly differ from a normal distribution (K-S test $D_{[10]}$= 0.21, p> 0.05; kurtosis= 0.25, skewness= 0.95).".

3.1 p4L3: 9:00 and 20:00: unclear, you mean that instantaneous values close to the daily mean were reached at these times of the day? Reword.

Response: Have reworded the sentence, as was also highlighted by RC1 above. "Reworded sentence for clarity "For all three months in barley, maximum R_s was seen between 12:00-15:00, minimum around 05:00 and daily means at 09.00 and *ca.* 20.00."".

p4L18: "...shows that the shift [...] would be totally missed": This type of very straight conclusion would better fit in the following paragraph, where such things are plainly demonstrated.

Response: Have moved this sentence to P4 L20:

"There was a significant interaction between sampling hour and crop type ($F_{[23,568]}$= 3.40, p< 0.0001), and a further significant interaction between crop and month ($F_{[2,568]}$= 202.44, p< 0.0001), emphasising that it is not at all valid to assume that measurements made in the adjacent two crops at the same time were sufficient for comparisons of total R_s flux.

Questions must be raised regarding the validity of using blanket, common sampling strategies to compare R_s between different vegetation types, given the marked diurnal changes in R_s demonstrated here. Indeed, if a protocol were employed which used the same sampling hour were used over several months, the significant interaction between shows that the shift from higher R_s in the *Miscanthus* in May to higher fluxes from the barley in June and July would be totally missed."

3.2 p5L1/Fig.5: Make clear that the lag shown in the figure for each months is the one that yielded the optimal $\mathbf{R^2}$ after experimentally testing all lag times in a range from x to y in steps of z (here and/or near p3L25 in 2.2).

Response: Amended the caption: "and the lag times shown for each month are those which yield the closest relationship (highest $\mathbf{R^2}$)".

p5L3-7: Although this hypothesis is plausible for your case, little is presented to support or falsify it. If radiation data are experimentally shifted to improve $\mathbf{R^2}$, so should be temperature data to check for the effect of the mentioned lagged response by improper temperature measurement depth (ideally it would have been measured at more than 1 depth, see comment on p2L30). The physically most consistent way to do so would be by Fourier analysis, since heat transport in the soil would introduce different delays for temperature variations on different temporal scales (e.g. diurnal cycle vs. slower or faster variations), but if variability in a certain time-window is strongly dominated by the diurnal cycle, a simple shifting might do as well. Also, the sentence is very long. Its 2nd half is unclear to me and should be reworded. It seems that a single case study, where hysteresis in the Rs-T relation could be attributed to photosynthates after comprehensive measurements, is used to infer that the same is true in your case. At the same time, an abundance of literature is ignored which demonstrates that also heat transport and measurement depth effects alone can cause hysteresis (e.g. Pavelka et al., 2007, Plant Soil 292:171 and Graf et al., 2008, Biogeosciences 5:1175 to mention just the earliest systematic studies, many follow-ups have been already mentioned by other reviewers).

Response: We refer to the revised Results and Discussion section. These papers are now included amongst other citations (see response to SC4, below) and we feel a balanced interpretation of our data has been presented. We acknowledge several papers (Pavelka et al. 2007; Graf et al. 2008; Oikawa et al 2014; Ruehr et al. 2010; Riveros-Iregui et al. 2007, Philips et al. 2011) which discuss hysteresis of R_s and soil temperature, but we also provide several examples of lagged R_s which suggest an effect of photosynthate (Xu and Qi 2001; Valdocchi et al. 2006; Gavrichkova and Kuzyakov 2008; Kuzyakov and Cheng 2004; Heinemeyer et al. 2011; Barron-Gafford et al. 2011; Zhang et al. 2015). We further reiterate that while we propose an explanation for the observations presented here, a definitive explanation was beyond the remit or expectation of this study (P6 L3).

4 p5L32: Specify what exactly (e.g. the ratio or difference in total repiration between two treatments) can be incorrect by 40 % - the way it is written now suggests that conclusions are, but what would be a 40 % incorrect conclusion?

Amended this sentence: “by as much as 40% relative to the respective daily means”.

Short comment #4 (SC4) Q. Zhang

The paper is a nice work for guiding soil respiration measurement design. Since the temperature response of soil respiration is so important to your topic, I assume the widely reported soil respiration-temperature hysteresis should be addressed. And you did discuss a little in 3.2 Environmental control of Rs, however, I think this section could be discussed even better by incorporating the knowledge from a few previous efforts. Please see follows. For the diurnal scale soil respiration-temperature hysteresis, there are a few representative works, including the classic Phillips et al. (2011) paper that applies mathematical models answering a few fundamental questions, like how soil temperature measurement depth selection, heat flow influence the respiration-temperature relation, etc; Afterwards, Zhang et al. (2015) combined both model exercise and field experiments to give a more comprehensive explanation of the occurrence and mechanism of the hysteresis. To exclude the possible effect of temperature depth selection by plotting respiration and temperature colocated at the same depth, this work demonstrated how heat flow, gas diffusion, photosynthesis contribute to the hysteresis, and also explained how soil moisture modulates hysteresis magnitude. Actually, the hysteresis may be more widely reported than the authors realized, see the literature list that reported field measured soil respiration-temperature hysteresis in Zhang et al. (2015). As a useful knowledge to this manuscript, the argument that "Even the CO2 flux (F(z)) and the environmental conditions at the same depth can be out of phase, since the flux integrates sources from other depths, causing hysteretic loops" (Zhang et al., 2015) would help explain why the temperature-depth selection cannot avoid hysteresis. Another useful information for this manuscript is related to photosynthesis control on soil respiration. As photosynthesis has long been suggested as the determinant of soil respiration by providing respiration substrate (e.g., Kuzykov and Cheng, 2001; Kuzyakov and Gavrichkova, 2010), Zhang et al. (2015) suggested the time-delayed photosynthesis impact on soil respiration contribute to the ‘8’ shaped soil respiration temperature hysteresis, and altered the hysteresis direction (clockwise cycle, or counterclockwise cycle) under different time lag levels of transferring photosynthate from leaves to roots. But these are numerical modeling representations, Zhang et al. (2015) also acknowledge more field validation are still required. The authors can think a little about this. A third representative and nice work is by Oikawa et al. (2014) as you cited. references:

Kuzyakov, Y., and W. Cheng (2001), Photosynthesis controls of rhizosphere respiration and organic matter decomposition, Soil Biol. Biochem., 33(14), 1915–1925.

Kuzyakov, Y., and O. Gavrichkova (2010), REVIEW: Time lag between photosynthesis and carbon dioxide efflux from soil: a review of mechanisms and controls, Global Change Biology, 16, 3386-3406.

Oikawa, P. Y., D. A. Grantz, A. Chatterjee, J. E. Eberwein, L. A. Allsman, and G. D. Jenerette (2014), Unifying soil respiration pulses, inhibition, and temperature hysteresis through dynamics of labile soil carbon and O_2, J. Geophys. Res. Biogeosci., 119, 521–536.

Phillips, C. L., N. Nickerson, D. Risk, and B. J. Bond (2011), Interpreting diel hysteresis between soil respiration and temperature, Global Change Biol., 17, 515–527.

Zhang, Q., G. G. Katul, R. Oren, E. Daly, S. Manzoni, and D. W. Yang (2015), The hysteresis response of soil CO_2 concentration and soil respiration to soil temperature, Journal of Geophysical Research-Biogeosciences, 120, 1605-1618.

We thank the reviewer for the additional references and we have incorporated them into a revised Results and Discussion section (P5 L4- P6 L5).

Technical Note: Differences in the diurnal pattern of soil respiration under adjacent *Miscanthus x giganteus* and barley crops reveal potential flaws in accepted sampling strategies.

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Abstract. For convenience, measurements used to compare soil respiration (R_s) from different land uses, crops or management practices are often made between 09:00-16:00, convenience which is justified by an implicit assumption that R_s is largely controlled by temperatureFor convenience, measurements used to compare soil respiration (R_s) from different land uses, crops or management practices are often made between 09:00-16:00, with an implicit assumption that R_s is largely controlled by temperature. Three months' continuous data presented here show distinctly different diurnal patterns of R_s between barley (*Hordeum vulgare*) and *Miscanthus x giganteus* (*Miscanthus*) grown on adjacent fields. whereas in *Miscanthus* after an initial early evening decline, R_s increased above the daily average during the night and in July maximum daily rates of R_s were seen at 22.00 and was significantly correlated with earlier levels of solar radiation, probably due to delays in translocation of recent photosynthateMaximum R_s in barley occurred during the afternoon and correlated with soil temperature, whereas R_s peaked in *Miscanthus* during the night and was significantly correlated with earlier levels of solar radiation, probably due to delays in translocation of recent photosynthate. Since the time of the daily mean R_s in *Miscanthus* occurred when R_s in the barley was 40% greater than the daily mean, it is vital to select appropriate times to measure R_s especially if only single daily measurements are to be madeSince daily mean R_s in *Miscanthus* coincided with levels 40% greater than the mean in barley, it is vital to select appropriate times to measure R_s if only single daily measurements are to be made.

Keywords

Soil respiration, *Miscanthus x giganteus*, barley, diurnal patterns, photosynthesis, carbon dioxide (CO_2), greenhouse gas (GHG), solar radiation, PAR

1 Introduction

Soil respiration (R_s) is a major process in the global carbon (C) cycle, contributing approximately 30% of ecosystem respiration (Bond-Lamberty and Thomson, 2010). Though the controls on R_s are less-well described than for photosynthesis, as atmospheric carbon dioxide (CO_2) concentrations pass 400 ppm it is becoming increasingly important to improve our understanding of this important biological process. The implications that changes in R_s might have for climate change have long been discussed (Schlesinger and Andrews, 2000) and in recent years the attention given to the potential of soils to sequester large amounts carbon to mitigate rising levels of atmospheric CO_2 through management practices (e.g. Gattinger et al., 2012) demands that we measure all aspects of the global carbon cycle, including R_s, as accurately as possible.

The most common method used to measure R_s is the closed chamber technique (Mosier, 1989) with manual chambers tending to be employed for sampling from a weekly to monthly basis (e.g. Drewer et al., 2012; Toma et al., 2011; von Arnold et al., 2005). R_s is generally accepted to be largely controlled by soil temperature (Bond-Lamberty and Thomson, 2010) and if combined with an assumption that soil temperature will be consistent across a single site, a logical expectation might be that the diurnal variation in R_s will also be consistent at that site. Many studies consider it sufficient to use a single simultaneous daily measurement of R_s to test for differences between different land uses or vegetation types and to extrapolate long-term budgets, (e.g. Barrena et al., 2013; Finocchiaro et al., 2014; Gauder et al., 2012; Johnson et al., 2010; Shvaleva et al., 2014; von Arnold et al., 2005; Zhang et al., 2013).~~The most common method used to measure R_s is the closed chamber technique (Mosier, 1989) with manual chambers tending to be monitored from a weekly to monthly basis (e.g. Drewer et al., 2012; Toma et al., 2011; von Arnold et al., 2005). R_s is generally accepted to be largely controlled by soil temperature (Bond-Lamberty and Thomson, 2010) with the consequence that many studies consider it sufficient to use a single simultaneous daily measurement of R_s to test for differences between different land uses or vegetation types and to extrapolate long term budgets, (e.g. Barrena et al., 2013; Finocchiaro et al., 2014; Gauder et al., 2012; Johnson et al., 2010; Shvaleva et al., 2014; von Arnold et al., 2005; Zhang et al., 2013).~~ Whilst the importance of selecting appropriate and synchronous sampling times is commonly recognised, measurement "windows" often vary across two hours (Kessavalou et al., 1998; Zhang et al., 2013) to as much as seven (Finocchiaro et al., 2014) or even eight hours (Gao et al., 2014), generally between 09:00-16:00; however, none of these cited studies provided any data to support these windows which are largely based on minimising time delays between comparisons and assumptions that minimised temperature changes are the key to measurement parity. Although work has been undertaken to ascertain the most suitable time of day to sample R_s manually (e.g. Savage and Davidson, 2003; Wang et al., 2012), these studies have focussed on a single vegetation type or land use thus do not resolve the issue of selecting the most appropriate sampling time at which to make comparisons between different experimental treatments or crops.

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In the current work the aim was to compare the R_s fluxes between two adjacent crops, as part of a fuller quantification of ecosystem C budgets. The two crops monitored in this study were the conventional arable crop barley (*Hordeum vulgare*), the second most widely planted arable crop in the UK (DEFRA, 2014), and the perennial grass species *Miscanthus x* *giganteus* (henceforth *Miscanthus*), which is increasingly cultivated as an energy crop. In this study the use of automated chambers allowed the collection of near-continuous measurements of R_s and the resulting data set was used to investigate the effect of sampling time and crop on R_s, and how this might differ across a period of several months.

2 Methods & materials

2.1 Study site and experimental design

Soil respiration (R_S) was measured using automated chambers and infrared gas analysers (IRGA, Licor LI-8100-101A, Lincoln NE, USA) with multiplexers (Electronic workshops, Department of Biology, University of York, York UK) beneath a seven year-old stand of *Miscanthus* and an April-sown spring barley in adjacent fields on a farm in the east of the United Kingdom, with one IRGA and one multiplexer deployed in each crop (see Drewer et al., (2012) for a full site description). Chambers (n=6) were placed at random within separate plots at least 1.5 m apart in the two fields and so were treated as independent replicates; chambers were seated over PVC collars (diameter 20 cm, height 10 cm) which were inserted *ca.* 2 cm into the soil in order to minimise the effect of cutting fine roots (Heinemeyer et al., 2011) and these remained in situ throughout the study, which was undertaken from May to August 2013. The chambers were programmed to close for two minutes during measurement, with a 30 second 'dead band' to allow for mixing of the headspace, in a continuous cycle between chambers. Collars did not exclude roots and no above-ground vegetation was included. Soil temperature and moisture at 5 cm depth were also measured every 15 minutes adjacent to each chamber collar and averaged over hourly intervals using vertically-installed sensors Soil respiration (R_s) was measured using automated chambers and infrared gas analyser (IRGA, Licor LI-8100-101A, Lincoln NE, USA) with multiplexers (Electronic workshops, Department of Biology, University of York, York UK) beneath a seven year-old stand of *Miscanthus* and an April-sown spring barley in adjacent fields on a farm in the east of the United Kingdom (see Drewer et al., (2012) for a full site description). Chambers (n=6) were placed at random within separate plots at least 1.5 m apart in the two fields and so were treated as independent replicates; chambers were seated over PVC collars (diameter 20 cm) inserted *ca.* 2 cm into the soil which remained in situ throughout the study, which was undertaken from May to August 2013. Collars did not exclude roots and no above-ground vegetation was included. Soil temperature and moisture at 5 cm depth were also measured every 15 minutes adjacent to each chamber collar and averaged over hourly intervals (Delta-T DL2 and GP1 loggers, SM200 soil moisture probes and ST1 temperature probes; Delta-T, Cambridge UK), and hourly meteorological data (solar radiation, air temperature) were recorded onsite using a weather station (WP1, Delta-T, Cambridge UK).

2.2 Data processing and analyses

R_S fluxes were calculated as linear regressions of CO_2 concentration against time and corrected for volume and temperature using the manufacturer's software (see manufacturer's manual https://www.licor.com/documents/jtpq4vg358reu4c8r4id.pdf) and subsequent analyses were conducted using SAS 9.3 (SAS Institute, Cary NC USA)The chambers were programmed to close for two minutes during measurement, with a 30 second 'dead band' to allow for mixing of the headspace in a continuous cycle between chambers; fluxes were calculated as linear regressions of CO_2 concentration against time and corrected for volume and temperature using the Licor software (see Licor manual) and subsequent analyses were conducted using SAS 9.3 (SAS Institute, Cary NC USA). In the first instance the R_s flux data were hourly averaged for each of the individual three months of the study, but to enable diurnal patterns to be more clearly identified, deviation from the daily mean was ascertained by subtracting hourly fluxes from the daily mean R_s and the data for each month were subsequently averaged. Cumulative R_s fluxes were calculated by trapezoidal integration for each chamber within both crops and averaged to estimate the total flux; data were not gap-filled, instead where there were gaps in the data for one crop, the corresponding fluxes from the other were omitted from the calculation to estimate cumulative flux. This resulted in a loss of 15 days over the study period (five days in May, six in June and four in July) which represented a total coverage of 80%. . These estimates were then used to investigate the influence of sampling hour on the monthly cumulative estimate of R_s by comparing cumulative fluxes calculated using individual sampling hours (e.g. deriving a cumulative estimate of R_s by integrating only fluxes measured between 14.00 and 15.00) and those using all measurements for each month. The cumulative fluxes for the whole period were tested for normality using a Kolmogorov-Smirnov (K-S) test, but due to the size of the dataset this approach was unsuitable for the cumulative fluxes for sampling hour and instead limits of kurtosis and skewness of ± 2 were used as acceptable deviation from a normal distribution (Field, 2013). Differences in the whole-period cumulative flux were tested using one-way analysis of variance; the effect of crop, sampling hour and month were tested using a mixed-effects model accounting for the repeated estimated totals from each chamber for each month (PROC MIXED in SAS, using the 'repeated' statement and an autoregressive covariance structure)These estimates were tested for normality and differences in the whole-period cumulative flux were tested using one-way analysis of variance; the effect of crop, sampling hour and month were tested using a mixed-effects model accounting for the repeated estimated totals from each chamber for each month (PROC MIXED in SAS, using the 'repeated' statement and an autoregressive covariance structure) .

Ancillary environmental data (soil temperature, soil moisture, solar radiation and air temperature) were averaged hourly and over each month using the same method applied to fluxes of R_s. These hourly averaged data were used in regression models to explain the diurnal pattern in R_s, and more detailed analyses were undertaken by performing separate regressions with flux measurements taken during the typical daily measurement window (09:00-16:00) and outside of this window. A further analysis was completed by performing regressions of fluxes against 'lagged' measurements of solar radiation, i.e. the effect of prior levels of solar radiation on R_s was tested.

3 Results and discussion

At the start of the study period (May) R_s tended to be higher in the *Miscanthus* than the barley (Fig 1), but this reversed during June and higher fluxes of R_s were consistently seen under the barley until the end of July. Highest rates of R_s were seen in the barley during early July (*ca.* 1500 mg-CO_2 m^{-2} h^{-1}) and declined soon after, whereas R_s climbed steadily under the *Miscanthus* until it reached a maximum of *ca.* 800 mg-CO_2 m^{-2} h^{-1} towards the end of July (Fig 1).

The hourly monthly averaged fluxes revealed strong diurnal patterns for R_s in both crops (Fig. 2). For all three months in barley, maximum R_s was seen between 12:00-15:00, minimum around 05:00 and daily means at 09.00 and *ca.* 20.00. However, R_s changed distinctly in the *Miscanthus* across the three months of the study. The magnitude of the daily variation in R_s was remarkably different between the two crops (Fig. 2): for both barley and *Miscanthus* the daily minima were *ca.* 10 % below the daily mean across the study, but where the maxima in barley increased from *ca.* 15% in May, to 20% in June to as much as 40% above the daily mean in July, it declined in *Miscanthus* from 20% in May, through 15% in June and finally just 10% above the daily mean in July (Fig. 2). During May the daily pattern of R_s was similar for *Miscanthus* and barley but in June, although R_s peaked around 15:00, after initially declining it increased again so that for the period 20:00 to 04:00 was greater than the daily mean. This pattern for R_s changed again through July, when the lowest daily R_s was seen at 09:00 coinciding with the daily mean for barley, whilst R_s for *Miscanthus* did not increase above the daily mean value until 18:00 peaking at 21:00, as much as five hours later than the peak in the barley.

The data did not significantly differ from a normal distribution (K-S test $D_{[10]}= 0.21$, $p> 0.05$; kurtosis= 0.25, skewness= 0.95). Cumulative R_s flux was greater from barley over the entire study period ($F_{[1,8]}=6.62$, $p<0.04$), there was a strong and significant effect of the chosen sampling hour on that estimate ($F_{[23,568]}= 4.28$, $p< 0.0001$) and a resulting strong significant difference between monthly totals ($F_{[2, 568]}= 901.35$, $p< 0.0001$). There was a significant interaction between sampling hour and crop type ($F_{[23,568]}= 3.40$, $p< 0.0001$), and a further significant interaction between crop and month ($F_{[2,568]}= 202.44$, $p< 0.0001$), emphasising that it is not at all valid to assume that measurements made in the adjacent two crops at the same time were sufficient for comparisons of total R_s flux.

Questions must be raised regarding the validity of using blanket, common sampling strategies to compare R_s between different vegetation types, given the marked diurnal changes in R_s demonstrated here. Indeed, if a protocol were employed which used the same sampling hour over several months, the significant interaction between crop and month shows that the shift from higher R_s in the *Miscanthus* in May to higher fluxes from the barley in June and July would be totally missed. For example, considering only the measurements taken around 15:00 in this study, in May not only would the cumulative R_s from both crops be overestimated, it would be concluded that R_s from barley was higher than or the same as for *Miscanthus*, when that clearly is far from correct (Fig. 3). Over the entire study, measurements made singly at just 15:00 would further bias the conclusions, so that in July R_s from the barley would be overestimated by 40%, whilst there would be a slight underestimate from the *Miscanthus*, introducing the real possibility of not only exaggerating differences between crops, but also of creating artefactual differences simply resulting from the choice of a standardised measurement protocol.

Analysis of environmental variables showed that R_s in the barley was a function of soil temperature (Fig. 4). Soil temperature also had a strong positive effect on R_s (Fig. 4) in the *Miscanthus* between 09:00-16:00 but it did not explain the night-time fluxes. during which time R_s was strongly positively correlated with the level of solar radiation seen earlier in the day (Fig. 5). Several studies have ascribed such hysteresis or apparent asynchronous R_s response to soil temperature to a discrepancy between depth of R_s source and the measurement depth of soil temperature (e.g. Oikawa et al., 2014; Graf et al., 2008; Pavelka et al., 2007) and this explanation cannot be discounted for the response seen here in *Miscanthus* since this study is limited by soil temperature measurements at a single depth (5 cm). Soil moisture has also been proposed as the driver of temperature hysteresis (Ruehr et al., 2010; Riveros-Iregui et al., 2007), though our analysis did not find that relationship on a diurnal scale: multiple regression of R_s with soil temperature and soil moisture did not improve the explanation of the daily variation in R_s. There was a short period (19th – 22nd July) however, following two weeks without rain, when soil moisture dropped to a low of 0.16 m^3 m^{-3} in the arable crop and during this time R_s dropped considerably (Fig. 1). When heavy rainfall elevated soil moisture, rates of R_s increased again which would suggest there is a threshold above which soil moisture is not limiting, an effect similar to that described by Xu and Qi (2001).

Alternatively, if solar radiation is considered a proxy measurement of photosynthesis, the delay in response of R_s may be a function of photosynthate translocation to roots and the rhizosphere, which has been shown to be important to all component processes of R_s (e.g. Heinemeyer et al., 2012) and having witnessed such a lag in an oak savannah system, Baldocchi et al. (2006) propose a similar explanation. This is further supported by Gavrichkova and Kuzyakov (2008) who showed that under constant temperature a diurnal response in R_s will still be evident under maize (*Zea mays*) but not from unplanted controls, and another study which demonstrated that shading maize plants will reduce the diurnal pattern in R_s (Kuzyakov and Cheng, 2004). This suggestion is further strengthened as the delay observed in the current study increased as the *Miscanthus* crop grew taller; from six hours in May, to seven in June and ten in July. It is known that translocation is slower in taller vegetation and may also be slowed as transpiration increases (Kuzyakov and Gavrichkova, 2010), as would be expected later in the summer. An obvious physical difference between the two crops monitored in this study is that of size, with *Miscanthus* exceeding 3 m when fully grown and barley less than 0.5 m, so the speed of translocation in barley may be quicker and therefore the effect of photosynthesis in this crop is more confounded with soil temperature (Kuzyakov and Gavrichkova, 2010). Differences in the diurnal pattern of R_s have been demonstrated between grass species and mesquite trees in savannah ecosystems (Barron-Gafford et al., 2011), and again between grasses and forest soils (Heinemeyer et al., 2011) which both reflect the differences presented here of temperature decoupled peak in R_s under the taller trees occurring later in the day. Such a lag in R_s cannot be assumed under all tall vegetation however, as studies under maize and switchgrass (*Panicum virgatum*), which share the physiological traits of height and C4 photosynthesis with *Miscanthus*, demonstrated a clear diurnal relationship between R_s and soil temperature (Han et al., 2008; Huang et al., 2016).

A lack of consensus persists regarding the cause of these lags in R_s, a point acknowledged by Phillips et al. (2011) in a study which used computer modelling to attempt to interpret hysteresis, and their analysis led them to conclude that the phenomenon might possibly be due solely to physical, not biological processes. A more recent modelling study provided further explanation of how both photosynthate and soil moisture might affect observed hystereses (Zhang et al., 2015). On the balance of our analysis and the literature cited here, we are inclined to hypothesise that it is the former which drives the lag presented in our data. However, it should be reiterated that a definitive explanation of the drivers of R_s hysteresis was beyond the scope of the current study and further targeted experimental work should be implemented if this additional aim is to be achievedAt the start of the study period (May) R_s tended to be higher in the *Miscanthus* than the barley (Fig 1), but this reversed during June and higher fluxes of R_s were consistently seen under the barley until the end of July. Highest rates of R_s

were seen in the barley during early July (*ca.* 1500 mg m^{-2} h^{-1}) and declined soon after, whereas R_s climbed steadily under the *Miscanthus* until it reached a maximum of *ca.* 800 mg m^{-2} h^{-1} towards the end of July (Fig 1).

3.1 Diurnal pattern of R_s

The hourly monthly averaged fluxes revealed strong diurnal patterns for R_s in both crops (Fig. 2). Consistently, R_s peaked between 12:00-15:00, was lowest around 05:00 and daily means at 09.00 and *ca.* 20.00 for all three months in barley, but R_s changed distinctly in the *Miscanthus* across the 3 months of the study. The magnitude of the daily variation in R_s was remarkably different between the two crops (Fig. 2): for both barley and *Miscanthus* the daily minima were *ca.* 10 % below the daily mean across the study, but where the maxima in barley increased from *ca.* 15% in May, to 20% in June to as much as 40% above the daily mean in July, it declined in *Miscanthus* from 20% in May, through 15% in June and finally just 10% above the daily mean in July (Fig. 2). During May the daily pattern of R_s was similar for *Miscanthus* and barley but in June, although R_s peaked around 15:00, after initially declining it increased again so that for the period 20:00 to 04:00 was greater than the daily mean. This pattern for R_s changed again through July, when the lowest daily R_s was seen at 09:00 coinciding with the daily mean for barley, whilst R_s for *Miscanthus* did not increase above the daily mean value until 18:00 peaking at 21:00, fully five hours later than the peak in the barley.

Cumulative R_s flux was higher from barley over the entire study period ($F_{[1,8]}$=6.62, p<0.04), there was a strong and significant effect of the chosen sampling hour on that estimate ($F_{[23,568]}$= 4.28, p< 0.0001) and a resulting strong significant difference between monthly totals ($F_{[2, 568]}$= 901.35, p< 0.0001). There was a significant interaction between sampling hour and crop type ($F_{[23,568]}$= 3.40, p< 0.0001), emphasising that it is not at all valid to assume that measurements made in the adjacent two crops at the same time were sufficient for comparisons of total R_s flux. Indeed, if the same protocol were used over several months, the significant interaction between crop and month ($F_{[2,568]}$= 202.44, p< 0.0001) shows that the shift from higher R_s in the *Miscanthus* in May to higher fluxes from the barley in June and July would be totally missed.

Questions must be raised regarding the validity of using blanket, common sampling strategies to compare R_s between different vegetation types, given the marked diurnal changes in R_s demonstrated here. For example, considering only the measurements taken around 15:00 in this study, in May not only would the cumulative R_s from both crops be overestimated, it would be concluded that R_s from barley was higher than or the same as for *Miscanthus*, when that clearly is far from correct (Fig. 3). Over the entire study, measurements made singly at just 15:00 would further bias the conclusions, so that in July R_s from the barley would be overestimated by 40%, whilst there would be a slight underestimate from the *Miscanthus*, introducing the real possibility of not only exaggerating differences between crops, but also of creating artefactual differences simply resulting from the choice of a standardised measurement protocol.

~~3.2 Environmental control of R_s~~

~~Analysis of environmental variables showed that R_s in the barley was a function of soil temperature (Fig. 4). This was also true in the *Miscanthus* between 09:00-16:00 when soil temperature had a strong positive effect on Rs (Fig. 4) but it did not explain the night-time increase in R_s. Outside of this time window R_s was strongly positively correlated with the level of solar radiation seen earlier in the day (Fig. 5) and we suggest that solar radiation serves as a proxy measurement of photosynthesis, with the delay a function of photosynthate translocation to roots and the rhizosphere. Having witnessed a similar such lag in an oak savannah system, Baldocchi et al. (2006) propose a similar explanation. Although there is the potential that a discrepancy between depth of R_s source and the measurement depth of soil temperature (5 cm) might explain the asynchronicity, however, hysteresis between response of R_s to soil temperature across many depths has been shown (e.g. Oikawa et al., 2014) and would suggest that such a response as seen in *Miscanthus* in this study is controlled by something other than soil temperature. This is further supported by the study of Gavrichkova & Kuzyakov (2008) which showed that under constant temperature a diurnal response in R_s will still be evident under maize (*Zea mays*) but not from unplanted controls, and another study which demonstrated that shading maize plants will reduce the diurnal pattern in R_s (Kuzyakov and Cheng, 2004). This suggestion is further strengthened as this delay increased as the *Miscanthus* crop grew taller; from six hours in May, to seven in June and ten in July. It is known that translocation is slower in taller vegetation and may also be slowed as transpiration increases (Kuzyakov and Gavrichkova, 2010), as would be expected later in the summer. An obvious physical difference between the two crops monitored in this study is that of size, with *Miscanthus* exceeding 3 m when fully grown and barley less than 0.5 m, so the speed of translocation in barley may be quicker and therefore the effect of photosynthesis in this crop is more confounded with soil temperature (Kuzyakov and Gavrichkova, 2010). Differences in the diurnal pattern of R_s have been demonstrated between grass species and mesquite trees in savannah ecosystems (Barron-Gafford et al., 2011), which reflect the differences presented here of temperature decoupled peak in R_s under the taller mesquite trees occurring later in the day. Such a lag in R_s cannot be assumed under all tall vegetation, however, as studies under maize and switchgrass (*Panicum virgatum*), which share the physiological traits of height and C4 photosynthesis with *Miscanthus*, demonstrated a clear diurnal relationship between R_s and soil temperature (Han et al., 2008; Huang et al., 2016)~~.

4 Conclusions

In this study strong, clear diurnal patterns in R_s have been demonstrated, and these are not consistent between different crops, even at a single location. Without the use of an automated flux measurement system, this discrepancy would not have been identified, although it is acknowledged ~~however it is acknowledged~~ that manual sampling techniques have an important role to play particularly when cost of equipment and access to power are a common limitation. It is therefore a matter of great importance that sampling strategies founded upon single daily measurements of R_s are undertaken at a time representative of the daily mean flux, and in order to do so it is absolutely vital that a thorough understanding of the diurnal variation is used to guide any sampling strategy. It is therefore suggested that especially in manual sampling experimental designs, the diurnal pattern of R_s is first established by measuring across a full 24 hour cycle and that this is revised periodically, since it has been shown here that the diurnal cycle may change greatly over several months. Failure to do so may lead to inaccurate long term estimates, and in experimental contrasts it may cause grossly incorrect (by as much as 40% relative to the respective daily means) conclusions to be drawnFailure to do so may lead to inaccurate long term estimates, and in experimental contrasts it may cause grossly incorrect (by as much as 40%) conclusions to be drawn. Since R_s is such a critical component of the global carbon cycle, it is essential that our understanding of this process, and how it is effected affected by management practices, be founded upon accurate data, which will only be achieved through well planned sampling strategies.

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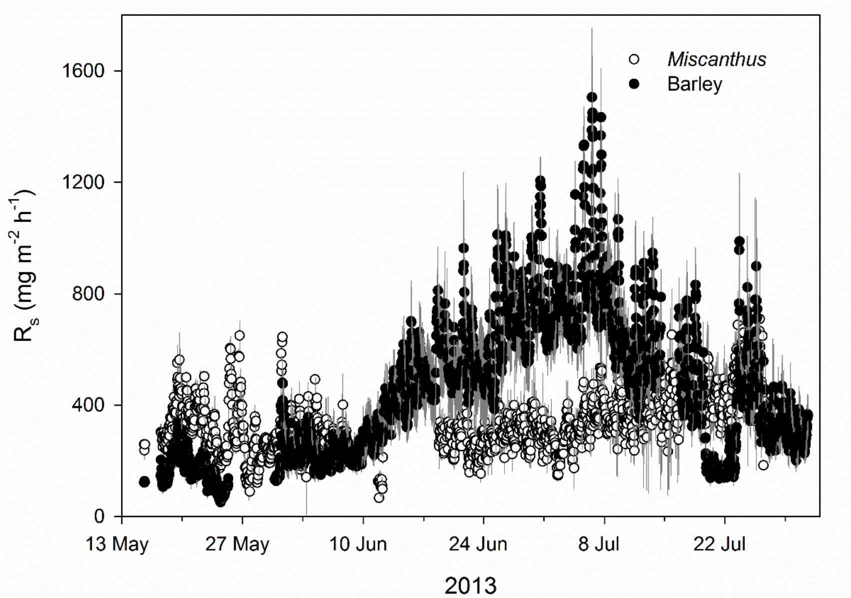


Miscanthus
Barley
R_s (mg m^{-2} h^{-1})
1600
1200
800
400
0
13 May
27 May
10 Jun
24 Jun
8 Jul
22 Jul
2013

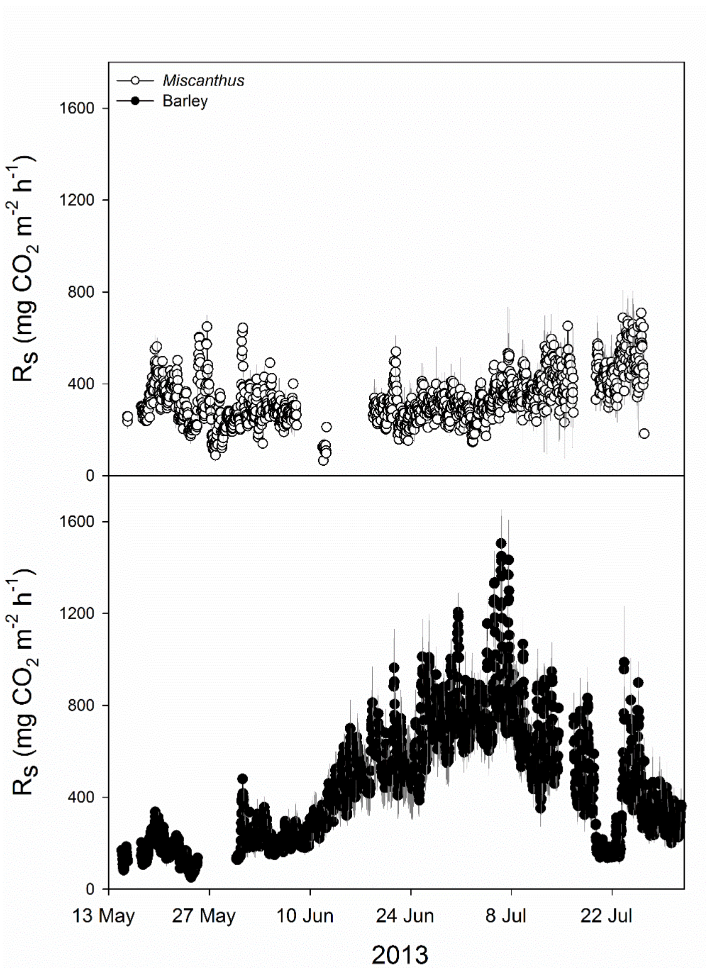
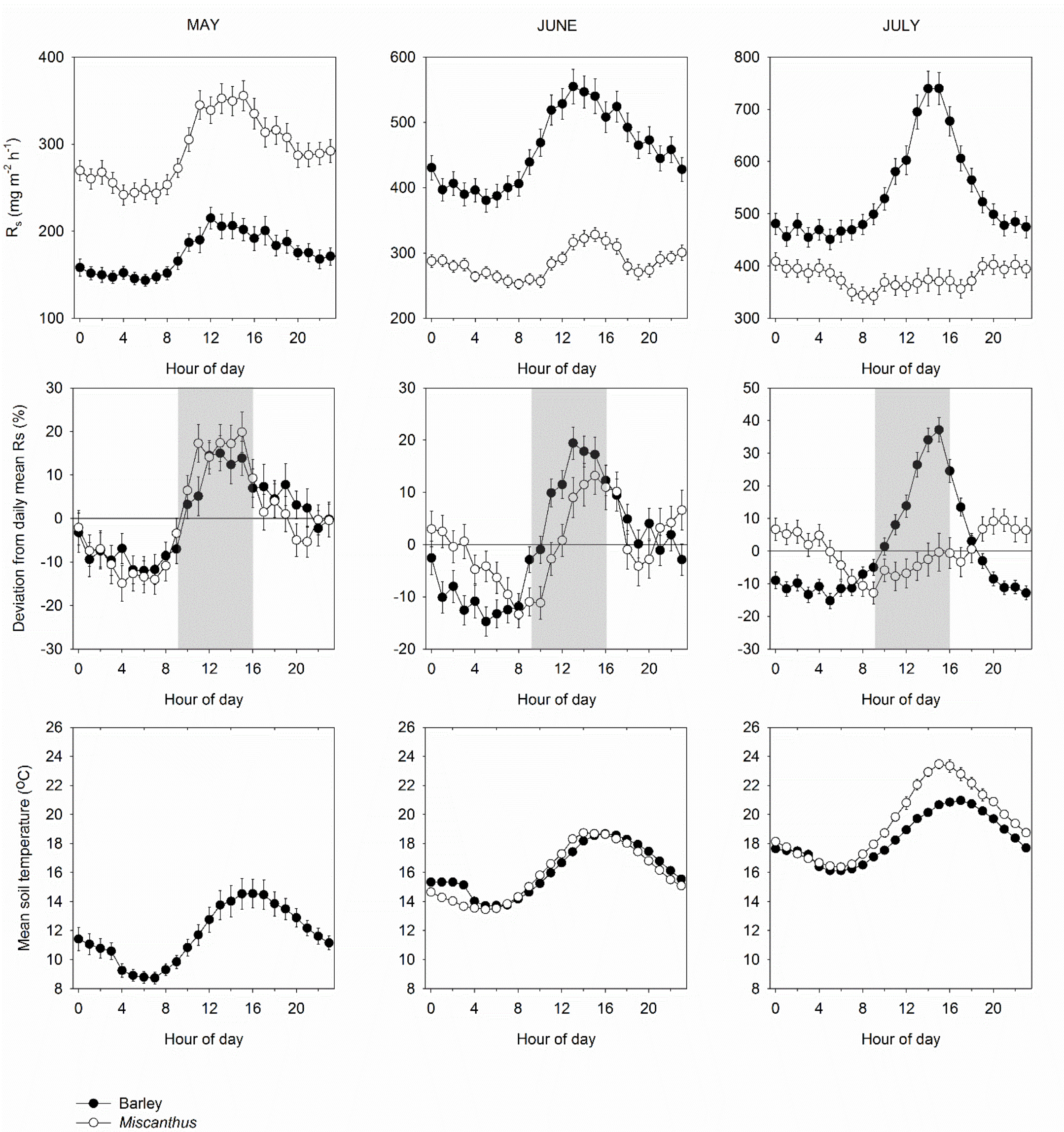


Figure 1. Mean (± 1SE, n=6) R_s from under *Miscanthus* and barley crops during summer 2013, measured using Licor automatic flux chambers.



MAY
JUNE
JULY
Rs (mg m-2 h-1)
Deviation from daily mean Rs (%)
Mean soil temperature (ºC)
Hour of day
Barley
Miscanthus

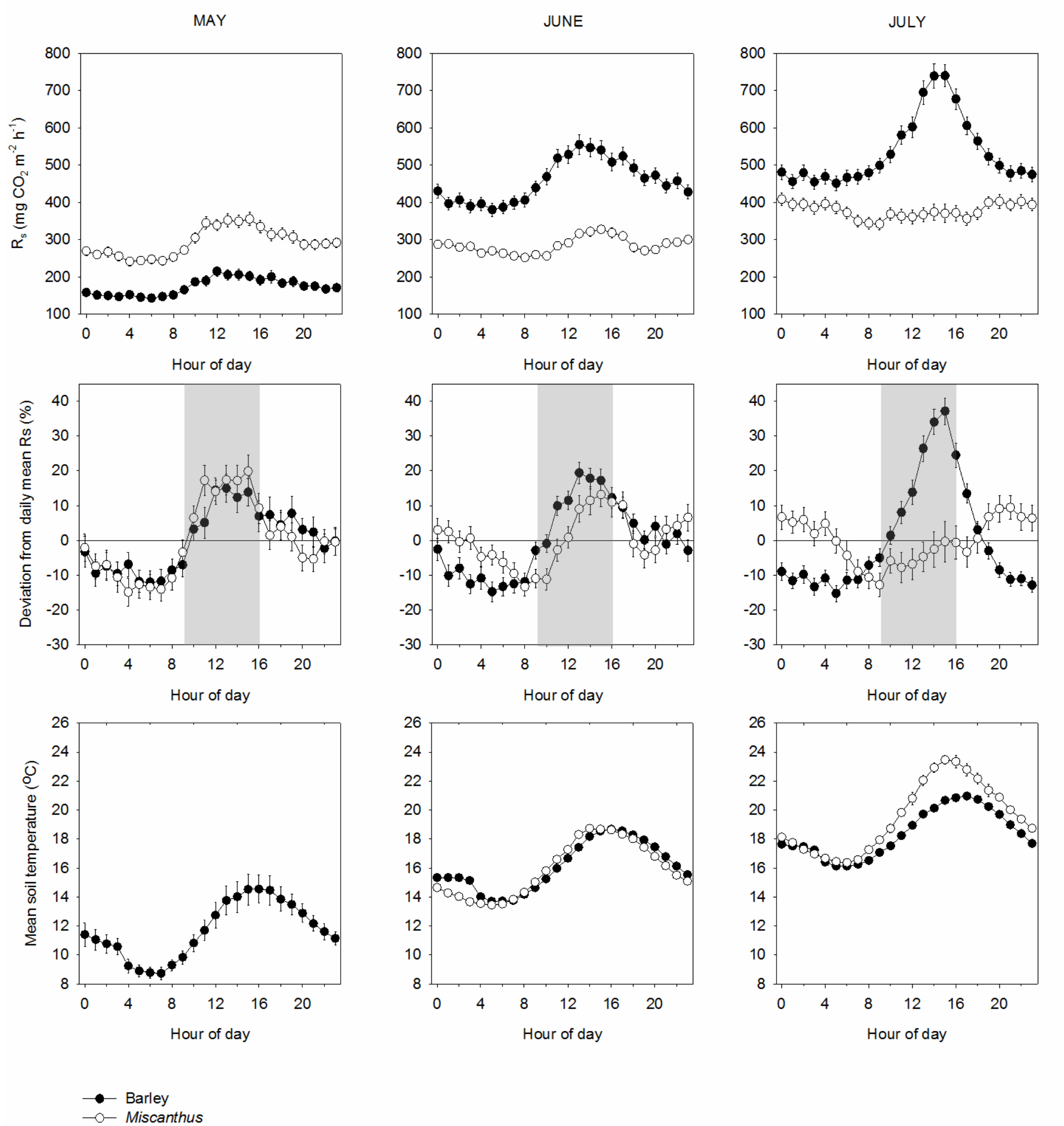


Figure 2. The diurnal pattern of R_s **and soil temperature at 5 cm depth** for each month of the study for barley and *Miscanthus* crops. Values shown are mean (± 1SE) average hourly absolute values of flux R_s (top row) and deviation from the daily mean (middle row). The shaded area of the middle panels represents the typical measurement window during which

manual sampling would take place. Zero deviation represents the daily mean flux, positive deviation representing fluxes greater than the mean and negative fluxes smaller than the mean.

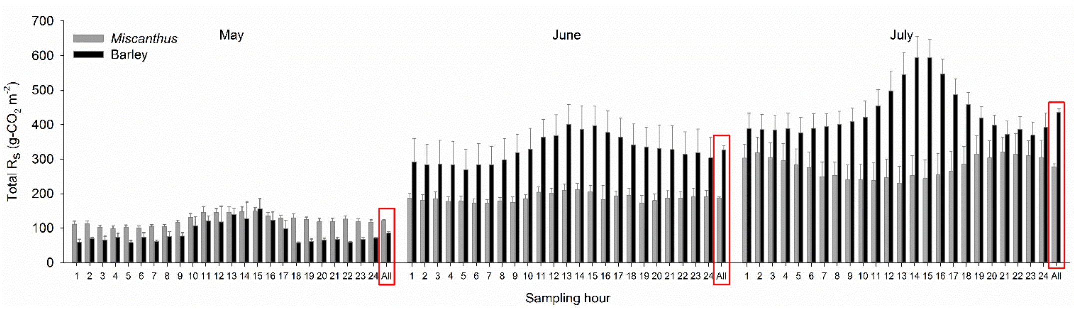
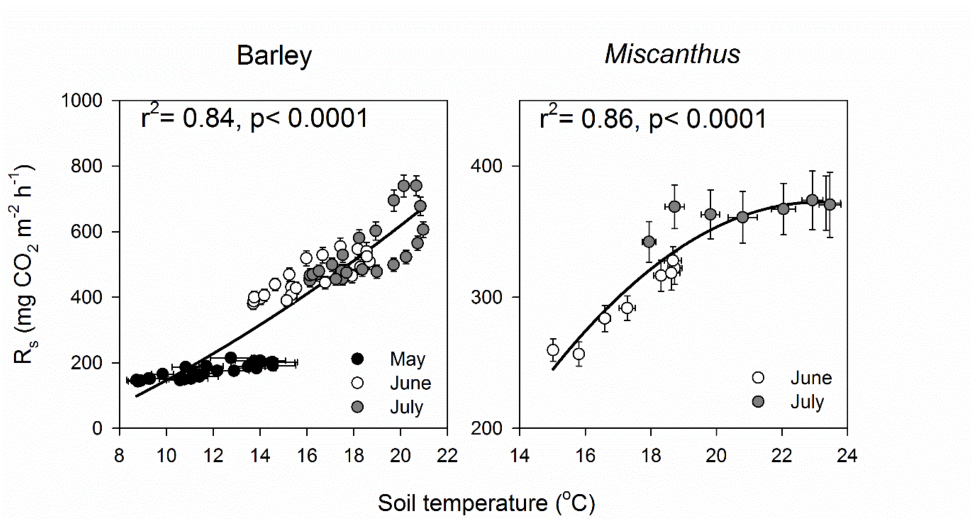


Figure 3. Estimates of the cumulative flux R_s under *Miscanthus* and barley crops using measurements taken using only single hours (1-24) or continuous measurements (All) across three months in summer 2013. Values shown are mean cumulative flux (± 1SE, n=6).



Barley
Miscanthus
r^2= 0.84, p< 0.0001
r^2= 0.86, p< 0.0001
R_s (mg CO_2 m^{-2} h^{-1})
May
June
July
June
July
Soil temperature (^oC)

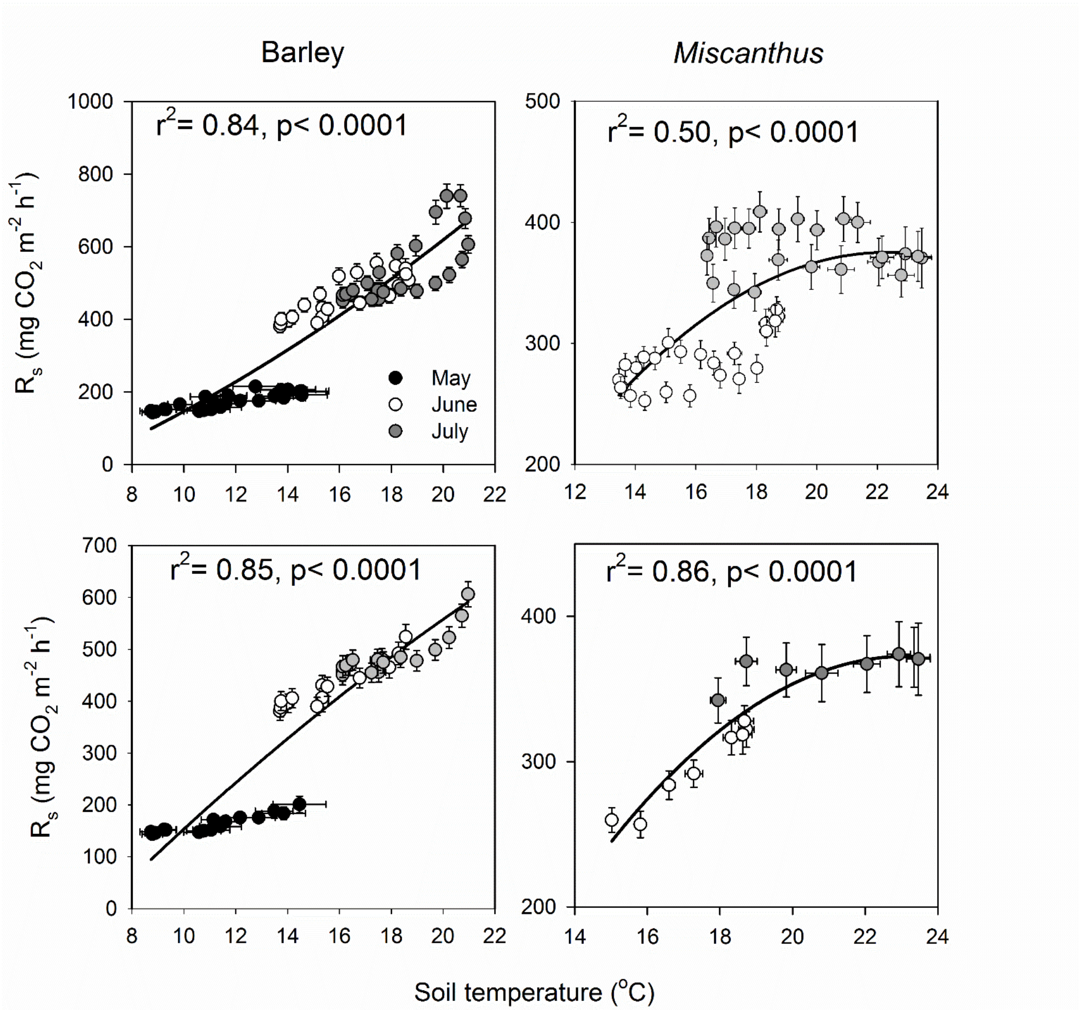


Figure 4. Regression models of monthly mean average hourly (± 1SE, n=6) flux R_S and soil temperature at 5 cm depth for barley (left column) and *Miscanthus* (right column). Data shown include full 24 hour period (top row) and only data from the typical manual measurement window of 09:00- 16:00 (bottom row). Soil temperature data were not available for *Miscanthus* during May.Regression models of monthly mean average hourly (± 1SE, n=6) flux R_S and soil temperature at 5 cm depth for barley (left panel) and *Miscanthus* (right panel). Data shown include full 24 hour period for barley, but only data from the typical manual measurement window of 09:00- 16:00 (see text) for *Miscanthus*. Soil temperature data were not available for *Miscanthus* during May.

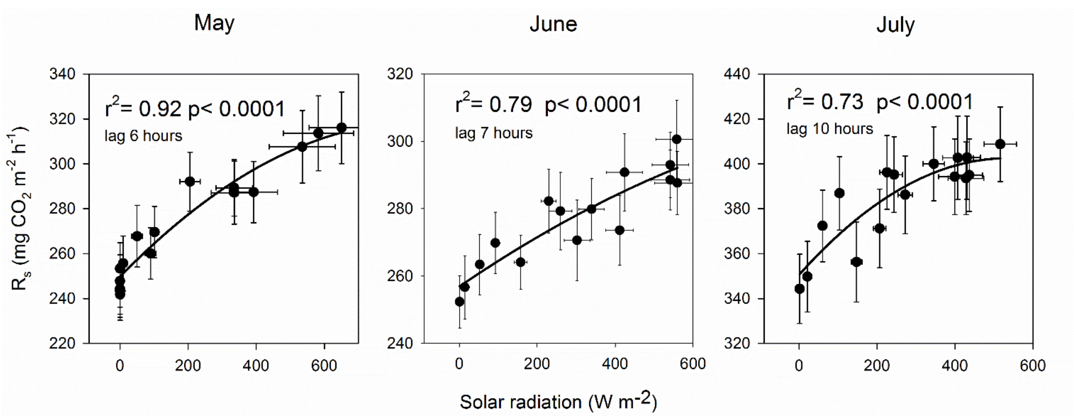


Figure 5. Response of R_s to preceding levels of solar radiation in *Miscanthus* outside of the typical manual measurement window (see text). Values shown are hourly means (±1SE n=6) averaged over each month. The lag time is the length of the offset between the measured solar radiation and the R_s, e.g. for May the relationship shown is that of solar radiation at 12.00 and R_s measured at 18.00 (lag time= 6 hours) and the lag times shown for each month are those which yield the closest relationship (highest R^2).Response of R_s to preceding levels of solar radiation in *Miscanthus* outside of the typical manual measurement window (see text). Values shown are hourly means (±1SE n=6) averaged over each month. The lag time is the length of the offset between the measured solar radiation and the R_s, e.g. for May the relationship shown is that of solar radiation at 12.00 and R_s measured at 18.00 (lag time= 6 hours).

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