Author's response to Reviewer 2 for Manuscript egusphere-2025-2849: "The Use of Newly Assimilated Photosynthates by Soil Autotrophic and Heterotrophic Respiration on a Diurnal Scale"

We thank Reviewer 1 for the valuable comments. We address all comments below and document the resulting changes to the manuscript. The reviewer's comments are shown in plain typeface, while the author's responses are highlighted in blue. Revised or added text appears in red. All line numbers refer to the original submitted work. Citations within this response are listed in the References section at the end of the document.

Response to Reviewer #2

1. This manuscript presents an investigation into the diurnal coupling between photosynthesis and soil respiration components (autotrophic - Ra, heterotrophic - Rh) in a pine forest. The use of cospectral analysis to explore time lags is a relevant approach. The authors found a strong diurnal link between Rh and GPP/PAR with a consistent 2-4 hour lag, contrasted with weaker/more variable links for Ra, which is potentially significant for understanding rapid C cycling. However, the manuscript in its current form has substantial weaknesses in clarity, methodology description, figure interpretation, and discussion depth that prevent publication.

Major revisions are required.

Response 1: We thank the reviewer for the constructive comments.

Major comments:

2. The separation standards regarding campaigns (especially C3, C4, and C5, C6) is valid and severe. The manuscript fails to adequately define what these campaigns represent (e.g., different seasons? specific meteorological conditions?), and why they were chosen? The method for partitioning soil CO2 efflux is fundamental to the entire study's conclusions but is either missing or described insufficiently. Without this, the validity of the Ra and Rh data, and thus the core findings, is questionable.

Response 2: We have expanded the description of measurement protocols and data classification to address these points.

- First, we partitioned soil CO_2 efflux into component fluxes, autotrophic (Ra) and heterotrophic respiration (Rh), using the root exclusion method. A deep PVC collar (35 cm depth) was inserted to sever roots, and the efflux 3-6 months following root severing was considered as Rh. The Ra was estimated by the difference between total soil CO_2 efflux and Rh. This explanation is provided in lines 86-103.

- Second, the available data of high-quality GPP, SR, Rh, PAR, Ts, and VWC data were divided into “campaigns” and “active” and “dormant” seasons based on GPP and SR flux magnitudes and temperature (as explained in lines 102-105 and expanded lines 105-106, as below). Specifically, the transition between C3 and C4 was based on a change in GPP (Fig. 2A) and between C5 and C6 based on SR. These transitions were also marked by temperature dynamics, in the case of C5 and C6 by deepening drought.

  “The categorization into seasons was based on physiological state, including canopy leaf area index (LAI), GPP, and SR values, as well as soil temperature and moisture conditions. Importantly, the vegetation was active throughout the year, and the “dormant” periods were characterized merely by lower GPP (and LAI and SR), not their cessation.”

- Aware that the data aggregation period may affect the results of the spectral analysis, we also analyzed the data without separating these periods (e.g., combining C3+C4 and C5+C6). The results showed similar cospectral peaks and similar patterns in lag times between respiration components (Ra and Rh) with potential drivers (GPP, PAR, Ts, and VWC). Only the standard deviations were larger with the longer averaging periods. Therefore, we chose to subdivide the data into 6 instead of 4 campaigns, as the differences in flux magnitudes may also signify changes in underlying physiology. We have added the explanation, after line 129, as follows:

  “Similarly, the spectral analyses were completed for data where campaigns C3–C4 and C5–C6 were not separated into active and dormant periods. The results showed similar cospectral peaks and similar patterns in lag times between respiration components (Ra and Rh) with potential drivers (GPP, PAR, Ts, and VWC). Only the standard deviations of time lags were larger with the longer averaging periods. Therefore, we chose to subdivide the data into 6 instead of 4 campaigns, as the differences in flux magnitudes may also signify changes in underlying physiology.”

3. Figures 3-7 are currently incomprehensible to the reader, as noted. The primary issue is the lack of explanation for the "Period" axis. This presumably represents the period of the cyclic components identified by the cospectral analysis (e.g., Wavelet Coherence? Cross-Wavelet Transform? Other?). How were the parameters chosen (e.g., wavelet type, wavelet power) in the cospectral analysis. Without this fundamental explanation in the caption, methodology, or axis label, the figures convey no meaningful information for general readers. The x-axis labels in Figs 6 & 7 are absent. Furthermore, the meaning of "cospectral peak" and "lag" in the context of these figures needs clearer explanation.

Response 3:

- We have clarified the “Period” axis by adding more explanation in the figure caption. Specifically, the “Period” axis represents the time frequency domain corresponding to time intervals from 6 hours to 64 days for this analysis. Also, we have added more detailed explanations in the Methods and figure legends on which transformation type was used: wavelet transformation was applied for a single time series (e.g., Rh, Ra), whereas cross-wavelet transformation was applied for the relationship between two time series (e.g., Rh vs GPP). Below is the revised Fig. 4 caption as an example:

  ”Figure 4. Average wavelet power in the frequency domain (Period; time intervals from 6 hours to 64 days) generated from the wavelet transformation of heterotrophic respiration (Rh; A–F) for six campaigns (C1–C6) at US-CRK. Average wavelet power in the frequency domain generated from the cross-wavelet transformation of heterotrophic respiration (Rh) against gross primary productivity (GPP; G–L), photosynthetically active radiation (PAR; M–R), soil temperature (Ts_5; S–X), and volumetric water content (VWC_5; Y–A4) at 5-cm depth for six campaigns at the US-CRK site. The bold contours indicate areas with significant coherence at the 5% level against white noise.”

- Regarding the parameters, we expanded the information after lines 140-141 as below. Please also refer to response #3 to reviewer #1.

  *“*The statistical significance of WT and XWT analyses was evaluated within the cone of influence (COI) at a 5% significance level using Monte Carlo methods (100 simulations). The surrogate data for significant analysis was generated using white noise (the color of the noise has little impact on the results; Grinsted et al., 2004; Vargas et al., 2010).“

- Thank you for pointing that out. We will add the x-axis title to Figs. 6 and 7.
- “Lag” refers to the time lag between the two time series, and we explain how it was derived in lines 137-139. A “spectral” or “cospectral peak” refers to the maximum wavelet power identified in the spectral or cospectral analysis.

4. The concluding statement ("These findings highlight the tight coupling between plant carbon status and soil microbial activity...") is not directly supported by the data presented. The study measures Rh, which includes microbial respiration, but it does not measure any specific microbial activity parameters (e.g., biomass, composition, enzyme activity, substrate use efficiency). Attributing the Rh signal directly to "soil microbial activity" without this link is speculative. The mechanism proposed (direct exudation of

recent assimilates) is plausible but remains inferred, not proven, by the Rh-GPP lag correlation.

Response 4: We define in the Introduction that, following earlier similar interpretations (e.g., Jilling et al., 2025; Meier et al., 2017; Mencuccini & Hölttä, 2010; Yang et al., 2022), we will consider Rh as an indicator of microbial activity. It could be argued that it is a better integrator of metabolic activity than the metrics listed by the reviewer above, although they, too, have been used for this purpose. While Rh does not tell whether the respiration was associated with growth or maintenance, much less with any specific metabolic step, it is well-suited for the current analysis. Our findings of (1) consistently strong cospectral peaks of Rh and GPP (or PAR), and (2) a consistent lag-lead relationship between Rh and GPP (or PAR), suggest consistent coupling between fresh carbon inputs and microbial activity on a diurnal scale. We expanded a paragraph starting from line 282 to explain the logic of this argument, as shown below.

> "The observed 2–4 hour lag of Rh relative to GPP at the diurnal scale is consistent with previously reported rates of pressure-concentration wave propagation in the phloem (Mencuccini & Hölttä, 2010) and the subsequent release into the rhizosphere (Kuzyakov & Gavrichkova, 2010). Although we did not directly measure exudate composition or microbial community responses, prior studies suggest that such rapid microbial utilization of new carbon inputs is facilitated by readily available substrates, such as soluble sugars and amino acids, that are tightly coupled with photosynthetic dynamics (Canarini et al., 2019). These labile compounds can activate microbes (Cheng et al., 2014; Kuzyakov & Blagodatskaya, 2015), and they can metabolize the compounds within hours (Kuzyakov & Gavrichkova, 2010). Therefore, we interpret it as a change in C availability (C status) in roots, with a likely pulse of exudation that triggered an increase in Rh within hours of enhanced photosynthetic activity, even though the mass flow of assimilates may occur over longer timescales (Liesche et al., 2015)."

5. The Discussion primarily restates results without sufficient context or mechanistic depth. Crucially, it lacks comparison with previous studies. How do the observed lags (2-4 hours for Rh-GPP) compare to other forest ecosystems? Are they faster/slower? How do they align with known phloem transport speeds or exudation dynamics reported elsewhere? The discussion of Ra's inconsistency is underdeveloped. What does "carbon availability from local starch reserves" mean mechanistically? Why would this buffer Ra differently than Rh? The dismissal of temperature needs more nuance – why might the lag vary? Could other factors (e.g., moisture, labile C pulses) interact with temperature differently at different times?

Response:

- Phloem mass flow occurs on the scale of 0.2 m/hr for gymnosperms (Liesche et al., 2015), whereas pressure concentration waves travel orders of magnitude faster. Moreover, the pressure concentration wave travel speed seems to increase with path length (lag time is almost independent of path length; Mencuccini and Hölttä (2010)).
- To our knowledge, the study by Yang et al. (2022) is the only one that has analyzed the response signatures separately for Ra and Rh. As noted in the paper, they observed a consistent lag of Rh relative to PAR by 2-6.5 hours across a year in a subtropical evergreen forest, which is similar to our results. Like our current report, Yang et al. (2022) was also based on flux measurements and did not include direct measurements of root exudation, microbial biomass, or enzyme activity.
- We have expanded the explanation on "carbon availability from local starch reserves", by modifying lines 275-277:

  "This suggests that tissue carbon status may have been buffered by starch reserves, as hydrolysis of stored starch can supply soluble sugars to meet the local energy and material demands (Zweifel et al., 2021)."

- Current results do not tell us why Ra is not tightly coupled to GPP. There could be several regulatory mechanisms, but as described above (modified from lines 275-277), we hypothesize that the starch reserves in the roots (both coarse and fine) act as a local buffer regulating cellular homeostasis and their metabolic needs. Given that the signaling of the metabolic state and energy needs occurs within individual cells, starch hydrolysis may be controlled at a rate that does not result in phloem loading, and thus does not contribute to exudation. Temperature and moisture may well modulate the different physiological processes, as also indicated by the slight variations in the mean lag times between Rh and GPP (point #3 in response to Review #2's question #2). However, the current dataset is insufficient to quantitatively resolve such interactions.

References

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