Responses to the comments of Referee 1

This manuscript addresses an original and stimulating question: whether carbon originating from belowground pools may contribute directly to the synthesis of a core photosynthetic biomolecule, chlorophyll a, in vascular plants. The use of the bomb-^{14}C transient combined with compound-specific radiocarbon analysis of pigments extracted from archival herbarium leaves is technically ambitious and conceptually clever.
The key observation, that Δ^{14}C of chlorophyll a (measured as pheophytin a) is consistently lower than Δ^{14}C of bulk leaf tissue across several decades, is intriguing and deserves attention. The study has the potential to open an important discussion about overlooked carbon pathways in plants.

>> We thank Referee 1 very much for the thoughtful review and constructive suggestions. We revised the manuscript in accordance with the comments and suggestions.

However, in its current form, the manuscript over-interprets what the dataset can uniquely demonstrate. Several alternative mechanisms, well documented in plant physiology and carbon allocation studies, can produce ^{14}C depletion in specific compounds without requiring direct incorporation of very old soil carbon. In addition, important methodological limitations significantly weaken the strength of the inference. Half of the dataset does not meet the authors' own purity criteria for pigment isolation, and the manuscript does not quantify the actual mass and radiocarbon signature (F^{14}C) of the carbon contamination introduced by the full analytical protocol. Without this information, it is difficult to assess how much of the observed ^{14}C depletion could arise from methodological contamination versus a true biological signal.

I therefore recommend major revision.

>> The major criticisms raised by Referee 1 and our corresponding responses are summarized as follows. Please note that both referees commented on almost the same points, so our responses to the comments of each referee are overlapping.

- **Quality assessment**: the impact of uncertain blank carbon on the results of Chl *a* Δ^{14}C and the subsequent mixing model (Comments 1 and 4)
    - We conducted a rigorous blank assessment for the entire procedure and found that the procedural blank has 0.32 ± 0.10 µgC. Even in the most extreme case where the wet chemistry blank Δ^{14}C was –1,000‰, the effect of the procedural blank on Chl *a* Δ^{14}C correction is smaller than the AMS analytical error (± 8‰, 1σ).
- **Methodological limitation**: the unclear effect of compound degradation during the storage and the experiment on the results (Comment 2)
    - We acknowledge that Chl *a* degradation occurs, however, there is no effect on Δ^{14}C values because isotopic fractionations associated with degradation are corrected by δ^{13}C, following reporting conventions.
- **Insufficient discussion** on plant physiology including the phytol salvage pathway (Comment 3)
    - We elaborated on the discussion regarding this topic in the revised manuscript. We also carried out a sensitivity analysis by exploring variations in the leaf turnover time, and these did not change our conclusions.
- **Overdiscussion** regarding the implications for global carbon cycle (Comment 5)
    - We agree that, in the absence of additional studies that reinforce our findings and support its broader relevance, we remove the discussions concerning the global-scale perspective and focus more on the interaction between the biosphere and the rhizosphere in the revised manuscript.

Please see our responses to the specific comments below. One new table, ten new figures (as Figures 2, S1, S2, S8, S9, S21, S22, S23, S24, and S25; the numbers reordered, and Table S1), and one MS Excel spreadsheet (Supplemental_Data.xlsx).

1) Pigment purity and contamination: a critical issue

Four of the eight pigment isolates (1952, 1965, 1966, 1968) fall outside the authors' own C/N-based purity threshold, with impurity carbon fractions estimated between 13% and 27%. These samples coincide with the period of maximum Δ¹⁴C separation between chlorophyll and bulk leaf — precisely where the interpretation is most sensitive.

The manuscript argues that remaining impurities are mainly triterpenoids and do not significantly affect conclusions. This is asserted rather than demonstrated quantitatively. A contaminant fraction of 15–25% is easily large enough to bias compound-specific Δ¹⁴C values at the scale discussed here. The mass and radiocarbon signature (F¹⁴C) of the contamination introduced along the analytical chain should be evaluated explicitly for each series of sample treatments. The procedure involves multiple solvent extractions, two HPLC separations, and an elemental analyzer prior to AMS measurement. Each of these steps can introduce carbon contamination or cross-contamination, whose magnitude may vary with solvent purity, column history, and instrument condition. For this reason, the effective procedural blank and its F¹⁴C value should be determined for each measurement batch rather than inferred from previous studies. This point is particularly critical here given the potentially far-reaching implications of the reported results.

Without this, the main inference rests heavily on data that are analytically fragile.

>> It was unfortunate that the four data in 1952, 1965, 1966, and 1968, the latter three of which show the greatest Δ^{14}C offset, were excluded from modelling due to their C/N ratios higher than the criterion. On the other hand, the impurity carbon percentage estimated using C/N ratios shows a good agreement with the triterpenoid carbon percentage estimated using GC/MS (please see also our response to your comment 4 and Table S1). This should not greatly influence the accuracy of the CSRA given that the triterpenoids are natural products originating from sample leaf matrix and were not extraneous to the sample (i.e., they do not derive from column bleed nor organic solvents that had been potentially made from fossil-fuel products being depleted in ^{14}C).

It should be mentioned that our analytical condition (solvent purity, column history, and instrument condition) was consistent across the batch of the samples. Furthermore, our additional assessment using HPLC/DAD and Orbitrap MS analysis indicate that no exogeneous compounds having absorbance at the 300–720 nm range or being ionized by electrospray ionization were found, respectively. Within these analytical windows, we do not see evidence for additional carbon impurities.

Although the Chl *a* standards that have modern ^{14}C (i.e., Δ^{14}C >0‰) and dead ^{14}C (i.e., Δ^{14}C ~ –1,000‰) are commercially unavailable, we estimated the size of excess carbon associated with the entire experimental and analytical procedure (hereafter procedural blank, PB) (see Workflow.pdf). The carbon mass (µgC) and Δ^{14}C (‰) of PB (M_{PB} and $\Delta^{14}C_{PB}$, respectively) are formulated as follows.

$$M_{PB} = M_A + M_B + M_C, \tag{R1}$$

$$\Delta^{14}C_{PB} = \frac{M_A \times \Delta^{14}C_A + (M_B + M_C) \times \Delta^{14}C_{B+C}}{M_A + M_B + M_C}, \tag{R2}$$

where M_A, M_B, M_C, $\Delta^{14}C_A$, and $\Delta^{14}C_{B+C}$ are the carbon mass (µgC) and Δ^{14}C (‰) of (A) Wet chemistry blank, (B) EA/AMS capsule blank, and (C) EA/AMS machine blank, respectively.

M_A and M_B were estimated as 0.04 ± 0.06 µgC and 0.08 ± 0.01 µgC, respectively, using nano EA/IRMS. In brief, 200 µL of trichloromethane (TCM) was added to the vial that passed through (A) wet chemistry procedure using a precleaned glass syringe, 20 µL of which was transferred into a pre-cleaned (washed with DCM three times) tin capsule (3 mm diameter, 6 mm height, and 25 µL volume, P/N 84.9906.26, Lüdi Swiss, Switzerland) using a pre-cleaned glass syringe on a hot plate set at 80 ºC. Once dried, the tin capsule was introduced to the autosampler of nano EA/IRMS. Using the regression line between the nano EA/IRMS peak areas and carbon amounts of known working standards (L-tyrosine: BG-T, Tayasu et al., 2011), the (A) Wet chemistry blank M_A and (B) EA/AMS capsule blank M_B values were quantified for the above dried capsules and blank tin capsules, respectively (Figure S21). Four repeated measurements were performed. For calculation details, see Supplemental_Data.xlsx.

The sum of M_B and M_C was estimated as 0.28 ± 0.08 μgC using MICADAS (Haghipour et al., 2019). $\Delta^{14}C_{B+C}$ was determined as –303 ± 24‰ by analyzing five blank tin capsules to achieve measurable carbon amounts (>1 μgC) using MICADAS (ETH analysis code 116866.2.1, $F^{14}C = 0.7034 \pm 0.0239$). The $\Delta^{14}C_A$ value is the only unknown in Equation R2, which might be derived from column bleed or organic solvents that had been potentially made from fossil-fuel products being depleted in ^{14}C. The $\Delta^{14}C_{Chl}$ value determined by MICADAS is represented using carbon mass (μgC) and $\Delta^{14}C$ of Chl *a*, impurity, and PB as follows.

$$\Delta^{14}C_{Chl} = \frac{(M_{Chl}+M_{Impurity})\times\Delta^{14}C_{Chl,corrected}+M_{PB}\times\Delta^{14}C_{PB}}{M_{Chl}+M_{Impurity}+M_{PB}}. \tag{R3}$$

Equation R3 is rewritten as follows.

$$\Delta^{14}C_{Chl,corrected} = \frac{(M_{Chl}+M_{Impurity}+M_{PB})\times\Delta^{14}C_{Chl}-M_{PB}\times\Delta^{14}C_{PB}}{M_{Chl}+M_{Impurity}}. \tag{R4}$$

We examined the effect of $\Delta^{14}C_A$ (i.e., wet chemistry blank) on the corrected $\Delta^{14}C_{Chl}$ values of the four Chl *a* data that satisfied the purity criterion and are used for modelling (1973, 1982, 1995, and 2007 CE, Table 1) (Figure S22). Even in the most extreme case where the $\Delta^{14}C_A$ was –1,000‰, the corrected $\Delta^{14}C_{Chl}$ values were within the MICADAS analytical error (± 8‰, 1σ) of their respective uncorrected $\Delta^{14}C_{Chl}$ values. Therefore, we concluded that the procedural blank does not analytically influence the observed $\Delta^{14}C_{Chl}$ values.

An aliquot (20 μL) of (A) Wet chemistry blank (dissolved in 200 μL TCM) was split into a pre-cleaned glass vial and dried. BSTFA was added to the vial and heated at 40 °C to derivatize the TMS ester. After drying with argon gas, the TMS ester was redissolved in 100 μL of dichloromethane and was injected (1 μL) to GC/MS with the same method being mentioned in the purity assessment. We found trace amounts of benzoic acid TMS derivative (Figure S23) and diethyl phthalate (Figure S24), which may have been derived from somewhere in the experimental procedure.

We addressed and elaborated upon the above statements in response to Referee 1's criticism more explicitly in the revised manuscript.

2) Use of pheophytin a as a proxy for chlorophyll a

Although the carbon skeleton of pheophytin a is identical to that of chlorophyll a, the issue is whether the fraction recovered after decades of degradation and multiple purification steps is chemically representative of the original chlorophyll pool present in the living leaf. Chlorophyll is known to undergo extensive degradation pathways, leading to numerous derivatives with different stability and solubility properties. The fraction ultimately isolated by HPLC may therefore represent a subset of molecules selected by their resistance to degradation and by the analytical procedure itself. This potential selection effect should be discussed, as the measured $\Delta^{14}C$ may reflect the properties of the surviving fraction rather than those of intact chlorophyll at the time of leaf formation.

>> We thank Referee 1 for pointing this out. As mentioned in Section 2.2 Preparation of chlorophyll *a* Lines 127–129 of the original manuscript, the Chl *a* concentration of our samples before fraction collection was roughly two-orders of magnitude lower than that of previous studies. The results suggest that a majority of the intact Chl *a* compound has been already degraded before sample collection as Referee 1 suggested. Furthermore, Chl *a* is sensitive to the presence of light and oxygen, which makes it unstable after extraction. This is the reason why we chose to acidify the extracts so that all Chl *a* present is converted to pheophytin *a* (Pheo *a*), which is more stable. We acknowledge, however, that this approach deprives us of the opportunity to discriminate Chl *a* and Pheo *a* as mentioned in Section 2.2, Lines 109–112 of the original manuscript. This is clearly one of the limitations in this study, and we addressed this issue in the revised manuscript.

However, Chl *a* degradation – both during the storage up to 70 years and during the experiment – should in theory have no impact Δ^{14}C results because any isotopic fractionation is internally corrected for using δ^{13}C values (Stuiver and Polach 1977). It should be mentioned that the Chl *a* survived after all is obviously intact because non-photoautotrophs such as fungi potentially colonizing the leaf surface during the storage in the herbarium cannot synthesize Chl *a*. We clarified this point in the revised manuscript.

3) Alternative explanations: stored carbon and internal recycling

The manuscript concludes that the additional carbon source must have a turnover time greater than 100 years and is therefore "most likely in the rhizosphere". This conclusion relies critically on the magnitude of the observed $\Delta^{14}C$ *offset between chlorophyll and bulk leaf. However, this offset is evaluated assuming a procedural contamination of only 0.1-0.2 µgC. If the effective contamination introduced along the analytical chain were larger than assumed, the true* $\Delta^{14}C$ *difference would be substantially reduced, potentially to values fully compatible with the mobilization of non-structural carbon that is few years old, especially in early season processes, rather than requiring an external millennial source. This possibility is not explored, yet it is central to the interpretation because the argument for an old rhizospheric source rests on the amplitude of the* $\Delta^{14}C$ *separation.*

In addition, chlorophyll metabolism includes known recycling pathways (notably involving phytol), which further complicates the assumption that chlorophyll carbon must derive only from current-year assimilates plus an external source.

>> The Chl *a* recycling pathway that salvages phytol should be a carbon source that is older than the current year photosynthates. However, such carbon is not sufficiently old to yield $\Delta^{14}C_{Chl}$ values lower than $\Delta^{14}C_{Atm(t)}$ unless the turnover time of the recycling pathway very long (>100 years), which is unrealistic considering the maximum age of the *Quercus* oak in frequently disturbed European forests (Martin-Benito et al., 2021) as also mentioned in Line 315 of the original manuscript. We made another figure illustrating the difference in turnover times of the carbon source within $\Delta^{14}C$ vs calendar year biplot space (Figure S2). The results were included in the revised manuscript.

We endeavored to characterize the origin of the contamination and have determined that it is derived mainly from triterpenoids (simiarenol and β-amyrin), which would not affect our main conclusions. We detailed our assessment as follows and in the Supplemental Information.

To assess the carbon amount of simiarenol and β-amyrin (both $C_{30}H_{50}O$, 84% carbon) contained in the 1952 and 1968 Pheo *a* samples, their standard materials of 90 µg and 100 µg, respectively, were weighed using a microbalance (MC5, Sartorius, Germany). These were then dissolved into 2,000 µL of dichloromethane to make their final carbon concentrations of 38 ngC μL^{-1} and 43 ngC μL^{-1}, respectively, and were injected (1 µL) to GC/MS. The peak areas at TIC were 104,533,222 and 142,408,002, which were then used to conversion coefficients for TIC peak area per ngC as 2,753,482 and 3,376,019 for simiarenol and β-amyrin, respectively. Since simiarenol and β-amyrin were the major impurities contained in the 1952 and 1968 Pheo *a* samples, respectively, the conversion coefficients were then applied to their respective TIC peak areas to derive 0.33 µgC and 0.43 µgC. These amounts divided by their respective start amounts (2.5 µgC and 3.8 µgC) yield the percentages of simiarenol and β-amyrin as 13% and 11% for the 1952 and 1968 Pheo *a* samples, respectively.

Finally, the percentages of simiarenol and β-amyrin based on the GC/MS analysis were compared with the impurity carbon percentages estimated by C/N and Equation (4) (13% and 16% for the 1952 and 1968 Pheo *a* samples, respectively). The differences between the two estimates (0.2% and 4.4% for the 1952 and 1968 Pheo *a* samples, respectively) were smaller than our purity criterion ($< 4.5\%$, 1σ) based on the C/N analytical error. Therefore, there is no evidence that the impurity in the purified Pheo *a* samples has carbon other than simiarenol and β-amyrin. The details of comparison between triterpenoid carbon and impurity carbon are summarized in Table S1.

If we consider alternative sources of contamination, such contaminants should have C/N ratios close to that of Chl *a* (11.8, w/w ratio) in order to satisfy this purity assessment criterion. The triterpenoids (simiarenol and β-amyrin, $C_{30}H_{50}O$) being detected on GC/MS as a major impurity have no nitrogen, which conflicts the above. Our purity assessment based on the C/N ratios might be unique in the CSRA research because its target compounds have been *n*-alkane, fatty acids, or GDGT with very high C/N ratios in most cases. However, we consider the C/N ratio approach used here as a very effective approach for purity assessment. Indeed, as we responded to the Referee 1's comment 1) and revised the manuscript, our approach can successfully prove that our data are analytically solid.

4) Mixing model and soil carbon endmember: underdetermined inference

The two-pool mixing model assumes atmosphere and rhizosphere as endmembers, but the rhizosphere carbon signature is not directly constrained. The inferred soil turnover time (≥ 1,300 years) is therefore strongly model-dependent. The heatmaps suggest that solutions are not uniquely constrained, and parameters appear sensitive to assumed leaf turnover times and endmember definitions.

In fact, the necessity to invoke such extremely old carbon pools is closely tied to the magnitude of the observed $\Delta^{14}C$ *offset and would become far less compelling if the effective procedural contamination were higher than assumed. A contribution from even modest amounts of* ^{14}C*-depleted carbon introduced during the analytical procedure would naturally push the model toward unrealistically old endmembers.*

Moreover, it is highly unlikely, from a soil biogeochemistry perspective, that vascular plants would access pedological carbon of such age. In temperate soils, carbon with turnover times on the order of a millennium typically corresponds to fractions that are strongly stabilized, often tightly associated with mineral surfaces and poorly accessible to microbial processing. Such pools are not readily involved in the dynamic exchanges that characterize mycorrhizal symbiosis and are therefore improbable candidates for contributing directly to metabolically active plant compounds.

>> We agree that the estimates of the underdetermined inference are model-dependent and sensitive to the $\Delta^{14}C$ offset between biosphere and rhizosphere. This is the reason why we used the historical leaf specimens for which the offset would be expected to be greater than for the present. Unfortunately, we decided not to use the data from the

1950s and 1960s samples whose offset was greatest due concerns about compound purity. Nevertheless the remaining four data points passed our purity criteria and were used for mutually independent model run.

We designed our indeterministic model not to deduce a unique algebraic solution from the differential equations, but to induce the most parsimonious and the least unlikely constraint from available data. Although the four years (1973, 1982, 1995, and 2007 CE) that met our purity criterion did not hold as large a $\Delta^{14}C$ offset as the other four years (1952, 1965, 1966, and 1968 CE), all of them showed consistent P_S values. Even if our estimates were affected by currently unconsidered factors, the most plausible P_S values would not be <10% in all the four years (Fig. 5) unless the soil turnover time T_S was extended to longer than 3,000 years, which is biogeochemically improbable. We acknowledge that the T_S values (1,000–3,000 years) were less constrained than P_S in our model, leaving a key question "how deep and old carbon in soils is incorporated into plants" open to debate as Referee 1 commented. This is a methodological limitation, which is inherently indeterministic due to the unavailable rhizospheric endmember. We detailed this point in the revised manuscript.

Our assumption of the leaf turnover time (T_Q, set as 1.5 years) refers to a previous study working on $\Delta^{14}C$ analysis of the *Quercus* oak (Ichie et al. 2013) as mentioned in Lines 210–211 of the original manuscript. We carried out a sensitivity analysis by tweaking the T_Q values from 0.5 to 5.0 years, and no substantial change was observed (Figure S25), suggesting that the model estimates are insensitive to the T_Q values at this range. It should be noted that the leaf turnover time ≥ 5.0 years is unlikely because such an endmember cannot explain the 1965 and 1966 Chl *a* $\Delta^{14}C$ data (Figure S2), which should be lower than the endmember $\Delta^{14}C$ to satisfy the mass balance. These results were added to the revised Supplemental Information.

We understand Referee 1's soil biogeochemistry perspective. The carbon pool with turnover times on the order of millennium is believed to be tightly stabilized by minerals and hardly accessed by plants or microbes. Therefore, such carbon is apparently the last candidate of a building block for the Chl *a* compound in *Quercus* oak among all other carbon available in the rhizosphere. The present study shows the very first but preliminary evidence of the millennial-aged carbon contributing to Chl *a*, which contrasts sharply with the current pedological paradigm. However, our observation is associated with analytical (lack of working standards) and methodological (unconstrained T_S values) limitations as mentioned earlier. Further investigations are needed to demonstrate the validity of our results, the potential significance of this process, and the broader relevance with respect to carbon cycling. We elaborated discussion in this point in the revised manuscript.

5) Overstated implications for global carbon budgets

The suggestion that this mechanism would “considerably revise” the terrestrial primary production carbon budget is not supported by the limited dataset and current level of uncertainty. While the observation is interesting, it does not yet justify global-scale implications.

>> We retracted the global-scale perspective and focus more on the interaction between the biosphere and the rhizosphere in the revised manuscript.

Reporting 14C data

I would also like to commend the authors for their careful respect of conventions in reporting physical quantities. The consistent use of significant figures, the correct spacing between numbers and units, and the proper notation for % and ‰ without unnecessary spacing are exemplary and, unfortunately, increasingly rare in the literature.

In the same spirit, it would be desirable to extend this rigor to the formulation of the Stuiver and Polach (1977) equation. The current presentation retains explicit “×100” or “×1000” factors in the expression of $\Delta^{14}C$*, which is not fully consistent with IUPAC conventions for the use of % and ‰ notation. Although this equation predates formal IUPAC recommendations, adopting the modern notation here would improve clarity and consistency with the otherwise very careful treatment of units throughout the manuscript. It* *would also be judicious to express $\Delta^{14}C$ explicitly from $F^{14}C$, following the format now widely recommended in the radiocarbon community (e.g., Reimer et al., 2004).*

$\Delta 14C = d14C - 2\cdot(d13C + 0.025)\,(1 + d14C)$ *with Δ14C, d13C and d14C expressed in ‰*

$\Delta 14C = F14C - 1$ *with Δ14C expressed in ‰*

Reimer, P.J., Brown, T.A. and Reimer, R.W. (2004) Discussion; reporting and calibration of post-bomb 14C data. Radiocarbon 46, 1299-1304.

>> We appreciate this comment. As Referee 1 mentioned, the radiocarbon content is first reported as $F^{14}C$. This dimensionless unit is useful for modelling in the present study, while it is not the most common notation in the field of Biogeosciences where the researchers generally prefer $\Delta^{14}C$ being expressed in ‰. Therefore, we decided to use $F^{14}C$ in the model and $\Delta^{14}C$ in everywhere else. $F^{14}C$ and $\Delta^{14}C$ are interchangeable, however, the equation suggested by Referee 1 is not strictly correct. The equation should be calibrated by the radioactive decay as follows (expressed in ‰).

$$\Delta^{14}C = F^{14}C \times e^{\{\lambda\times(1950-x)\}} - 1, \qquad \text{(R5)}$$

where λ and x are the decay constant of ^{14}C ($1/8267 = 1.21 \times 10^{-4}$) and the year when $^{14}C/^{12}C$ was measured, respectively. This comment reminded us that we also used a wrong conversion equation that did not consider the decay calibration. Hua et al. (2022) provided both $F^{14}C$ and $\Delta^{14}C$ of atmospheric CO_2 during 1950 and 2019, so we used their $F^{14}C$ in the model and $\Delta^{14}C$ in the figures. The re-running of the model using the correct equation resulted in the estimates being slightly changed; however, our main conclusions do not change. We updated the results in the revised manuscript.

Appendix

I may have overlooked a supplementary file, but I did not find an appendix providing the complete dataset underlying the measurements. For transparency and reproducibility, it will be necessary to include a table reporting all individual measurements, following *standard reporting practices for radiocarbon analyses. This should include, for each sample, the laboratory identification, the carbon mass submitted to AMS, the measured $F^{14}C$ values, and—consistent with the presentation in the manuscript—the corresponding $\Delta^{14}C$ values.*

>> We revised Table 1 to have all the primary information of the AMS results as suggested.

Table and Figure legends that were added to the revised manuscript and Supplemental Information

Figure 2. Workflow for Chl *a* sample preparation and its associated impurity and procedural blank assessments.

Tabe S1. Comparison between triterpenoid carbon and impurity carbon

Figure S1. Summary of key reactions in chlorophyll *a* anabolism and catabolism (Ischebeck et al. 2006; Vavilin & Vermaas 2007) highlighted in the present study.

Figure S2. The difference in turnover times of the carbon source on the $\Delta^{14}C$ vs. calendar year biplot space.

Figure S8. (a) Total ion chromatogram and (b) mass spectrum of the simiarenol standard. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S9. (a) Total ion chromatogram and (b) mass spectrum of the β-amyrin standard. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S21. The Faraday cup 2 (*m/z* 44, CO_2) peaks for 1 μgC working standard, AMS tin capsule, and procedural blank (ten times diluted) on overlaid nano-EA/IRMS chromatograms.

Figure S22. The effect of wet chemistry blank $\Delta^{14}C$ on the corrected $\Delta^{14}C_{Chl}$ values of the four Chl *a* data that satisfied the purity criterion and are used for modelling (1973, 1982, 1995, and 2007 CE, solid horizontal lines). Dashed lines represent the corrected $\Delta^{14}C_{Chl}$ value as a function of the wet chemistry blank $\Delta^{14}C$ (from –1,000 to 0‰) with the MICADAS analytical error (± 8‰, 1σ). The black vertical line and shading denote the AMS capsule and machine blank $\Delta^{14}C$ (–303 ± 24‰).

Figure S23. (a) Total ion chromatogram of the procedural blank (TMS reagent background subtracted) and (b) mass spectrum of benzoic acid TMS derivative (6.3 min).

Figure S24. (a) Total ion chromatogram of the procedural blank (TMS reagent background subtracted) and (b) mass spectrum of diethyl phthalate (10.3 min).

Figure S25. Sensitivity analysis of the two-pool mixing model for the change in its two outputs; the most plausible (a) proportion of soil carbon P_S and (b) turnover time of soil carbon T_S in response to the change in *Quercus* leaf turnover time T_Q values from 0.5 to 5.0 years. Mean and standard deviation of 1973, 1982, 1995, and 2007 data are shown.

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End of responses to the comments of Referee 1

Responses to the comments of Referee 2

The potential transfer pathways of carbon from the rhizophere into plants is a relevant subject of which the mechanisms and extent are not fully understood and which is of interest to readers of Biogeosciences. The approach to analyse archival herbarium leaves to compare the radiocarbon content of bulk leaves with Chl a (through pheophytin a) from the same leaves is original. Moreover, the finding that $\Delta^{14}C$ *values of chlorophyll a were found to be consistently lower than those of bulk leaves shows potential for a relevant contribution to the discussion on carbon transfer pathways.*

>> We thank Referee 2 very much for the thoughtful review and constructive suggestions. We revised the manuscript in accordance with the comments and suggestions.

However, due to methodogological uncertainties and the neglection of known alternative physiological mechansims, the manuscripts – in its current form – draws strong conclusions that go beyond what can be derived from the data analysis. Therefore I recommend major revision.

>> The major criticisms raised by Referee 2 and our corresponding responses are summarized as follows. Please note that both referees commented on almost the same points, so our responses to the comments of each referee are overlapping.

- **Quality assessment**: the impact of uncertain blank carbon on the results of Chl *a* Δ^{14}C and the subsequent mixing model (First comment)
    - We conducted a rigorous blank assessment for the entire procedure and found that the procedural blank has 0.32 ± 0.10 μgC. Even in the most extreme case where the wet chemistry blank Δ^{14}C was –1,000‰, the effect of the procedural blank on Chl *a* Δ^{14}C correction is smaller than the AMS analytical error (± 8‰, 1σ).
- **Methodological limitation**: the unclear effect of compound degradation during the storage and the experiment on the results (Second comment)

  - We acknowledge that Chl *a* degradation occurs, however, there is no effect on Δ^{14}C values because isotopic fractionations associated with degradation are corrected by δ^{13}C, following reporting conventions.
- **Insufficient discussion** on plant physiology including the phytol salvage pathway (Third comment)
  - We elaborated on the discussion regarding this topic in the revised manuscript. We also carried out a sensitivity analysis by exploring variations in the leaf turnover time, and these did not change our conclusions.
- **Overdiscussion** regarding the implications for global carbon cycle (Fourth comment)
  - We agree that, in the absence of additional studies that reinforce our findings and support its broader relevance, we remove the discussions concerning the global-scale perspective and focus more on the interaction between the biosphere and the rhizosphere in the revised manuscript.
- Please see our responses to the specific comments below. One new table, ten new figures (as Figures 2, S1, S2, S8, S9, S21, S22, S23, S24, and S25; the numbers reordered, and Table S1), and one MS Excel spreadsheet (Supplemental_Data.xlsx).

My major concerns are:

Δ14C values of chlorophyll a were found to be consistently lower than those of bulk leaves. However, four out of eight samples did not fall within the permissible impurity range set by the authors themselves. Eventhough the authors speculate about explanations and the respective samples were not used for the model estimations, carbon contamination during the lab analysis and its effect on the results was not assesed. This reduces confidence in the results and opens up the possibility for other explanatory mechanisms, which were not considered.

>> It was unfortunate that the four data in 1952, 1965, 1966, and 1968, the latter three of which show the greatest Δ^{14}C offset, were excluded from modelling due to their C/N

ratios higher than the criterion. On the other hand, the impurity carbon percentage estimated using C/N ratios and GC/MS analysis of triterpenoids shows a good agreement with the triterpenoid carbon percentage estimated using GC/MS (please see also our response to your comment 4 and Table S1). This should not greatly influence the accuracy of the CSRA given that the triterpenoids are natural products originating from sample leaf matrix and were not extraneous to the sample (i.e., they do not derive from column bleed nor organic solvents that had been potentially made from fossil-fuel products being depleted in ^{14}C). It should be mentioned that our analytical condition (solvent purity, column history, and instrument condition) was consistent across the batch of the samples. Furthermore, our additional assessment using HPLC/DAD and Orbitrap MS analysis indicate that no exogeneous compounds having absorbance at the 300–720 nm range or being ionized by electrospray ionization were found, respectively. Within these analytical windows, we do not see evidence for additional carbon impurities.

Although the Chl *a* standards that have modern ^{14}C (i.e., Δ^{14}C >0‰) and dead ^{14}C (i.e., Δ^{14}C ~ –1,000‰) are commercially unavailable, we estimated the size of excess carbon associated with the entire experimental and analytical procedure (hereafter procedural blank, PB) (see Workflow.pdf). The carbon mass (µgC) and Δ^{14}C (‰) of PB (M_{PB} and $\Delta^{14}C_{PB}$, respectively) are formulated as follows.

$$M_{PB} = M_A + M_B + M_C, \tag{R1}$$

$$\Delta^{14}C_{PB} = \frac{M_A \times \Delta^{14}C_A + (M_B + M_C) \times \Delta^{14}C_{B+C}}{M_A + M_B + M_C}, \tag{R2}$$

where M_A, M_B, M_C, $\Delta^{14}C_A$, and $\Delta^{14}C_{B+C}$ are the carbon mass (µgC) and Δ^{14}C (‰) of (A) Wet chemistry blank, (B) EA/AMS capsule blank, and (C) EA/AMS machine blank, respectively.

M_A and M_B were estimated as 0.04 ± 0.06 µgC and 0.08 ± 0.01 µgC, respectively, using nano EA/IRMS. In brief, 200 µL of trichloromethane (TCM) was added to the vial that passed through (A) wet chemistry procedure using a precleaned glass syringe, 20 µL of which was transferred into a pre-cleaned (washed with DCM three times) tin capsule (3 mm diameter, 6 mm height, and 25 µL volume, P/N 84.9906.26, Lüdi Swiss, Switzerland) using a pre-cleaned glass syringe on a hot plate set at 80 ºC. Once dried, the tin capsule was introduced to the autosampler of nano EA/IRMS. Using the regression line between the nano EA/IRMS peak areas and carbon amounts of known working standards (L-tyrosine: BG-T, Tayasu et al., 2011), the (A) Wet chemistry blank M_A and (B) EA/AMS capsule blank M_B values were quantified for the above dried capsules and blank tin capsules, respectively (Figure S21). Four repeated measurements were performed. For calculation details, see Supplemental_Data.xlsx.

The sum of M_B and M_C was estimated as 0.28 ± 0.08 µgC using MICADAS (Haghipour et al., 2019). $\Delta^{14}C_{B+C}$ was determined as –303 ± 24‰ by analyzing five blank tin capsules to achieve measurable carbon amounts (>1 µgC) using MICADAS (ETH analysis code 116866.2.1, $F^{14}C = 0.7034 \pm 0.0239$). The $\Delta^{14}C_A$ value is the only unknown in Equation R2, which might be derived from column bleed or organic solvents that had been potentially made from fossil-fuel products being depleted in ^{14}C. The $\Delta^{14}C_{Chl}$ value determined by MICADAS is represented using carbon mass (µgC) and $\Delta^{14}C$ of Chl *a*, impurity, and PB as follows.

$$\Delta^{14}C_{Chl} = \frac{(M_{Chl}+M_{Impurity})\times\Delta^{14}C_{Chl,corrected}+M_{PB}\times\Delta^{14}C_{PB}}{M_{Chl}+M_{Impurity}+M_{PB}}. \qquad \text{(R3)}$$

Equation R3 is rewritten as follows.

$$\Delta^{14}C_{Chl,corrected} = \frac{(M_{Chl}+M_{Impurity}+M_{PB})\times\Delta^{14}C_{Chl}-M_{PB}\times\Delta^{14}C_{PB}}{M_{Chl}+M_{Impurity}}. \qquad \text{(R4)}$$

We examined the effect of $\Delta^{14}C_A$ (i.e., wet chemistry blank) on the corrected $\Delta^{14}C_{Chl}$ values of the four Chl *a* data that satisfied the purity criterion and are used for modelling (1973, 1982, 1995, and 2007 CE, Table 1) (Figure S22). Even in the most extreme case where the $\Delta^{14}C_A$ was –1,000‰, the corrected $\Delta^{14}C_{Chl}$ values were within the MICADAS analytical error (± 8‰, 1σ) of their respective uncorrected $\Delta^{14}C_{Chl}$ values. Therefore, we concluded that the procedural blank does not analytically influence the observed $\Delta^{14}C_{Chl}$ values.

An aliquot (20 µL) of (A) Wet chemistry blank (dissolved in 200 µL TCM) was split into a pre-cleaned glass vial and dried. BSTFA was added to the vial and heated at 40 °C to derivatize the TMS ester. After drying with argon gas, the TMS ester was redissolved in 100 μL of dichloromethane and was injected (1 μL) to GC/MS with the same method being mentioned in the purity assessment. We found trace amounts of benzoic acid TMS derivative (Figure S23) and diethyl phthalate (Figure S24), which may have been derived from somewhere in the experimental procedure.

We addressed and elaborated upon the above statements in response to Referee 2's criticism more explicitly in the revised manuscript.

The implictations of measuring pheophytin a as proxy for Chl a are not discussed. How could pigment degradation due to long-term storage impact the results? How were the herbarium leaves stored and what could be the effect on the result?

>> As mentioned in Section 2.2 Preparation of chlorophyll *a* Lines 127–129 of the original manuscript, the Chl *a* concentration of our samples before fraction collection was roughly two-orders of magnitude lower than that of previous studies. The results suggest that a majority of the intact Chl *a* compound has been already degraded before sample collection as Referee 1 suggested. Furthermore, Chl *a* is sensitive to the presence of light and oxygen, which makes it unstable after extraction. This is the reason why we chose to acidify the extracts so that all Chl *a* present is converted to pheophytin *a* (Pheo *a*), which is more stable. We acknowledge, however, that this approach deprives us of the opportunity to discriminate Chl *a* and Pheo *a* as mentioned in Section 2.2, Lines 109–112 of the original manuscript. This is clearly one of the limitations in this study, and we addressed this issue in the revised manuscript.

However, Chl *a* degradation – both during the storage up to 70 years and during the experiment – should in theory have no impact $\Delta^{14}C$ results because any isotopic fractionation is internally corrected for using $\delta^{13}C$ values (Stuiver and Polach 1977). It should be mentioned that the Chl *a* survived after all is obviously intact because non-photoautotrophs such as fungi potentially colonizing the leaf surface during the storage in the herbarium cannot synthesize Chl *a*. We clarified this point in the revised manuscript.

A two pool mixing model was used to estimate the contribution and turnover time of the soil pool. The model assumes that Chl a only originates from current year photosynthates, which ignores known chlorophyll recycling pathways, while the less known pathway through soil carbon was much less constrained in the model. A would have at least expected a sensitivity analyses showing the implications of these assumptons.

>> The Chl *a* recycling pathway that salvages phytol should be a carbon source that is older than the current year photosynthates. However, such carbon is not sufficiently old to yield $\Delta^{14}C_{\mathrm{Chl}}$ values lower than $\Delta^{14}C_{\mathrm{Atm(t)}}$ unless the turnover time of the recycling pathway very long (>100 years), which is unrealistic considering the maximum age of the *Quercus* oak in frequently disturbed European forests (Martin-Benito et al., 2021) as also mentioned in Line 315 of the original manuscript. We made another figure illustrating the difference in turnover times of the carbon source within $\Delta^{14}C$ vs calendar year biplot space (Figure S2). The results were included in the revised manuscript.

Our assumption of the leaf turnover time (T_Q, set as 1.5 years) refers to a previous study working on $\Delta^{14}C$ analysis of the *Quercus* oak (Ichie et al. 2013) as mentioned in Lines 210–211 of the original manuscript. We carried out a sensitivity analysis by tweaking the T_Q values from 0.5 to 5.0 years, and no substantial change was observed (Figure S25), suggesting that the model estimates are insensitive to the T_Q values at this range. It should be noted that the leaf turnover time ≥ 5.0 years is unlikely because such an endmember cannot explain the 1965 and 1966 data (Figure S2), which should be lower than the endmember $\Delta^{14}C$ to satisfy the mass balance. These results were added to the revised Supplemental Information.

Eventhough a direct transfer of C from the rhizosphere to trees through fungal uptake of organic N has been observed in other studies, the mechanism remains speculative in the current study. The statement that the findings of this study would call for a considerable revison of the carbon budget and cycle is not supported by its results given its limited scale (4 samples, one species) and methodological uncertainties.

>> The present study shows the very first but preliminary evidence of the millennial-aged carbon contributing to Chl *a*, which contrasts sharply with the current pedological paradigm. However, our observation is associated with analytical (lack of working standards) and methodological (unconstrained T_S values) limitations as mentioned earlier. Further investigations are needed to demonstrate the validity of our results, the potential significance of this process, and the broader relevance with respect to carbon cycling.

We retracted the global-scale perspective and focus more on the interaction between the biosphere and the rhizosphere in the revised manuscript.

Table and Figure legends that were added to the revised manuscript and Supplemental Information

Figure 2. Workflow for Chl *a* sample preparation and its associated impurity and procedural blank assessments.

Tabe S1. Comparison between triterpenoid carbon and impurity carbon

Figure S1. Summary of key reactions in chlorophyll *a* anabolism and catabolism (Ischebeck et al. 2006; Vavilin & Vermaas 2007) highlighted in the present study.

Figure S2. The difference in turnover times of the carbon source on the $\Delta^{14}C$ vs. calendar year biplot space.

Figure S8. (a) Total ion chromatogram and (b) mass spectrum of the simiarenol standard. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S9. (a) Total ion chromatogram and (b) mass spectrum of the β-amyrin standard. The asterisk denotes the peak for which the mass spectrum is obtained.

Figure S21. The Faraday cup 2 (*m/z* 44, CO_2) peaks for 1 μgC working standard, AMS tin capsule, and procedural blank (ten times diluted) on overlaid nano-EA/IRMS chromatograms.

Figure S22. The effect of wet chemistry blank $\Delta^{14}C$ on the corrected $\Delta^{14}C_{Chl}$ values of the four Chl *a* data that satisfied the purity criterion and are used for modelling (1973, 1982, 1995, and 2007 CE, solid horizontal lines). Dashed lines represent the corrected $\Delta^{14}C_{Chl}$ value as a function of the wet chemistry blank $\Delta^{14}C$ (from –1,000 to 0‰) with the

MICADAS analytical error (± 8‰, 1σ). The black vertical line and shading denote the AMS capsule and machine blank Δ^{14}C (–303 ± 24‰).

Figure S23. (a) Total ion chromatogram of the procedural blank (TMS reagent background subtracted) and (b) mass spectrum of benzoic acid TMS derivative (6.3 min).

Figure S24. (a) Total ion chromatogram of the procedural blank (TMS reagent background subtracted) and (b) mass spectrum of diethyl phthalate (10.3 min).

Figure S25. Sensitivity analysis of the two-pool mixing model for the change in its two outputs; the most plausible (a) proportion of soil carbon P_S and (b) turnover time of soil carbon T_S in response to the change in *Quercus* leaf turnover time T_Q values from 0.5 to 5.0 years. Mean and standard deviation of 1973, 1982, 1995, and 2007 data are shown.

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End of responses to the comments of Referee 2