

Reviewer: Stefanie Lutz

The authors present an analysis of multi-scale monitoring in combination with CSIA to assess source apportionment and degradation of the herbicide S-metolachlor in a mesoscale agricultural catchment in France. They calculate that around 98% of S-metolachlor has been degraded over the 5-month growing season based on both their mass balance approach and CSIA. However, CSIA did not give a clear result regarding source apportionment between S-metolachlor from WWTPs and diffuse sources, respectively. The authors show that their CSIA methods can be a time and cost-efficient, yet reliable way to estimate pesticide degradation compared to a mass-balance approach requiring high-frequency measurements at different scales within a catchment.

General and specific comments

The study fits the scope of HESS. It makes a valuable contribution to the field of water quality and shows ways forward in catchment-scale monitoring. It is one of the first studies analysing the use of CSIA for assessing both sources and degradation of a pesticide. The authors present a substantial number of different methods and extensive monitoring data. Overall, the work is clear and described well in the paper. Thank you for the interesting read! See below for some specific comments on the text.

- The title: if you put it as a question, please add “does” or “can” for proper English grammar.

Thank you for the constructive feedbacks and comments.

We have slightly modified the title as follow:

“How does integrating multi-scale monitoring and compound-specific isotope analysis improve the evaluation of S-metolachlor degradation in agro-ecosystems?”

- Lines 76—77: These studies did not use isotope mixing models, at least if you think of the typical use of this term in literature. I'd prefer calling this isotope-fractionation reactive transport modelling, or reactive transport modelling including isotope fractionation or alike.

We have revised lines 77 to 79 in accordance with the reviewer's suggestion and now use the term “isotope-fractionation reactive transport modelling”.

“Moreover, isotope-fractionation reactive transport models based on CSIA data have been developed to support source apportionment at the hillslope scale (Lutz and Van Breukelen, 2014) and to predict pesticide biodegradation (Lutz et al., 2017).”

Aim (i) of the study: “evaluate the potential of CSIA data collected along the river network as a proxy for evaluating upstream topsoil degradation of S-metolachlor”. Only later in the text it

becomes clear to me why you are not looking at CSIA in the topsoil directly, but rather use the in-stream isotope data as a proxy. The reader will only find out in the methods that the latter has not been the original aim, but is a result of the strong matrix effects in the analysis. I would leave this aim more open, as reading this immediately raised questions regarding why you would not go for the isotope data from the topsoil directly. So something along the lines of CSIA data collected at different locations to evaluate topsoil and river degradation of S-metolachlor.

To follow the suggestion, we have now rephrased the aim (L85-90):

" The study aimed to (i) evaluate the potential of CSIA data collected along the river network to evaluate topsoil and river degradation of S-metolachlor, (ii) quantify the river network contribution to overall degradation at the catchment scale, and (iii) differentiate between pesticide sources, including diffuse agricultural applications and point-source inputs from wastewater treatment plants (WWTP). To achieve these objectives, a multi-scale sampling strategy was applied, integrating S-metolachlor mass balance, in-stream transit time analysis, and CSIA data from sources to the catchment outlet. "

- Figure 1: In this figure, A3 is not the outlet of the catchment. It is obvious that there will be little S-metolachlor added to the stream in the last bit, but I am wondering nonetheless why A3 does not coincide with the actual outlet of the catchment shown here.

We apologise for the confusion. A3 represents the outlet of the study catchment. The original delineation shown in Figure 1 extended beyond the actual catchment boundary to include downstream land use for comparative purposes (see comparison below). To avoid further misunderstanding, we have revised Figure 1 by clipping the land use map to the actual catchment boundary.



- Line 141: could you mention to what extent these three transformation products cover all possible degradation pathways for S-metolachlor? This might be interesting in the comparison of mass balances via CSIA, transformation products, and Eq. 6.

Among the numerous transformation products (TPs) identified (typically over 30, as reported by Steele *et al.*, 2008, <https://doi.org/10.2134/jeq2007.0166>), metolachlor ESA, OXA, and NOA are consistently described as the most prevalent in environmental waters, both in terms of frequency of detection and concentration (<https://doi.org/10.1016/j.scitotenv.2022.156696>, <https://doi.org/10.1021/acs.est.1c00466> and <https://doi.org/10.1007/s11356-025-35979-3>) and they have legal thresholds across for groundwater (European Commission , Off. J. Eur. Union, L 155, 127-175, 2011). These three TPs of metolachlor are those regulated in Europe (EU Regulation No. 546/2011).

We incorporated an independent dataset provided by the Rhin-Meuse Water Agency as supplementary evidence of ongoing degradation processes over the same period and in locations proximal to our sampling sites. However, the monthly grab-sampling protocol employed by the agency precluded accurate integration of TP concentrations into our mass-balance calculations. This limitation has been clarified in the revised manuscript (L145–L148):

“These three TPs do not encompass all possible degradation pathways of S-metolachlor; instead, they represent a subset of particular concern for groundwater used as a drinking resource. This concern arises from their high mobility, persistence, and are frequent detection across Europe (Baran et al., 2022; Menger et al., 2021; Pasquini et al., 2025). Moreover, each is subject to regulatory thresholds for groundwater quality (European Commission, 2011).”

And lines 155-157: “However, the monthly grab sampling protocol employed by the Water Agency, conducted without simultaneous discharge measurements, precluded accurate integration of TP concentrations into the mass balance calculations.”

- Lines 161-163: could you mention here what the range of timeframes covered by one composite sample is (roughly)? We can derive this from the data presented later, but it would be good to read about this here already.

Water samples, associated to flow proportional sampling were collected weekly and combined into composite samples. These composite samples represented water collected over a period ranging from 4.9 to 21 hours (22.8 ± 0.8 hours, $\bar{x} \pm SD$), determined according to hydrograph characteristics that distinguished between baseflow and high-flow conditions.

We have now incorporated this information in lines 167–170.: *“Water samples (up to twelve 330 mL glass bottles) were collected weekly and merged into composite samples, represented water collected over a 4.9 to 21-hour period (22.8 ± 0.8 hours, $\bar{x} \pm SD$). The sampling window was determined based on hydrograph characteristics, allowing separation of baseflow and high-flow conditions.”*

- Equation 3: why do you use instantaneous and not average water discharges in period i, similar to what is done for the concentrations?

We have clarified this in Line 232 to L236: *“S-metolachlor concentrations were obtained from composite water samples, representing period-averaged values. In contrast, discharge data were available at high temporal resolution and used in their instantaneous form. By pairing these representative concentrations with continuous flow measurements, we accounted for temporal variability in hydrological conditions, enabling a more accurate estimate of total pesticide mass export over the monitoring period, rather than isolated load snapshots.”*

- Lines 286-287: “2019 for the same month was the five time drier and three times warmer,...”. I do not understand – how did you determine this? What does three times warmer mean to you, for example?

The sentence has been corrected as follows (line 301 to 304): *“During the seven-month period from March to the end of September, 2019 was the fifth driest year in the past two decades, with total precipitation reaching 418 ± 79 mm (2000–2019 average). In terms of temperature, it was also the third warmest year, with a mean of 16.3 °C, compared to the 2000–2019 average of 15.7 ± 0.7 °C. These data are based on records from the Météo-France station in Entzheim, located approximately 10 km south of the catchment.”*

Lines 327-329: could you explain in more detail why the seasonal ESA to OXA ratio of 3.1 further supports the predominance of subsurface flow in the hydrological functioning of the Souffel catchment”? This is not clear to me.

Rose et al. (2018, <https://doi.org/10.1016/j.scitotenv.2017.08.154>) identified distinct patterns of ESA/OXA ratios according to the dominant hydrological pathway, i.e. runoff- or subsurface flow-dominated discharge, across a set of catchments. With an ESA/OXA ratio of 3.1, the Souffel catchment is classified, for the seven-month period in 2019, as subsurface flow-controlled, in accordance with the typology proposed by Rose et al. (see “Fig. 3. Mean percent MET, MESA and MOXA in water samples from the environmental compartments in the seven study areas. The shaded areas represent the estimated expected ratio of metolachlor to degrade based on an environmental compartment's flow path” in Rose et al., 2018). We have now clarified the link with Rose et al. conclusion, lines 345-348:

“The seasonal ESA/OXA ratio of 3.1, derived from mean concentrations of $56.8 \pm 12.3\%$ for ESA and $16.7 \pm 4.4\%$ for OXA across the eight Water Agency sites ($n = 88$; Table S13 in the Supplement), aligns with the distinct ratio patterns reported by Rose et al. (2018), which are indicative of dominant hydrological pathways. Specifically, this ratio supports the

predominance of subsurface flow in the hydrological functioning of the Souffel catchment."

- Lines 329-331: please correct this sentence.

We have re-written the sentence as in L349-350: *"During dry periods, higher electrical conductivity was observed at site A2 ($\sigma = 1.19 \pm 0.25 \text{ mS cm}^{-1}$) compared with site A1 ($\sigma = 0.58 \pm 0.31 \text{ mS cm}^{-1}$; $\bar{x} \pm SD$), suggesting mixing between groundwater and surface water."*

Figure 2: Could you add the rainfall in panel (a) also on the right side?

What do the colours in d represent?

I think in general, the legend could be a bit more clear. It took me some time to understand what I am seeing here. Maybe better to have a small inset in each panel so we know right away what we are looking at?

We thank the reviewer for the constructive suggestions regarding Figure 2. In response, we have revised the figure so that each panel now includes its own inset legend. We believe this substantially improves clarity compared to the previous single shared legend, allowing readers to more readily interpret the data.

Regarding the suggestion to add rainfall data to the right side of panel (a), we carefully considered this option. While we acknowledge the potential benefit of positioning rainfall directly above the discharge panel, duplicating the rainfall plot on both sides could create confusion, potentially leading readers to infer differences or additional information between the panels.

To avoid this ambiguity, we have opted to retain a single rainfall panel. We note, however, that major rainfall events are already indicated by dashed lines superimposed on the discharge curve, providing a concise and integrated visual reference without redundancy.

Lines 409-410: Could the authors briefly explain how they come to this finding? I assume it is because of the high export combined with low-intensity rainfall, but it would be good to explicitly mention this (briefly) here.

As Events 1 to 7 occurred shortly after S-metolachlor applications on sugar beet and maize plots, and a substantial residual mass was predicted to remain following these events (see Fig. S1 in the Supplement), we can reasonably conclude that the initial phase of S-metolachlor load dynamics was transport-limited. In contrast, the later phase, observed in July and August, was mass-limited, consistent with significant in situ biodegradation in the topsoil. A comparable shift from transport-limited to mass-limited behaviour over the

course of a growing season has been reported for various pesticides in a vineyard catchment (Imfeld et al., 2020).

In the revised draft, we provide a more detailed explanation of this process in Lines 425 to 431 as follows: *“Given the high and spatially uniform S-metolachlor load across the catchment (Fig. 1; Table S1 in the Supplement) and the timing of herbicide application, the mass reservoir during events 1 to 7 (up to 15 June) can be considered effectively semi-infinite relative to runoff volumes. This implies that once hydrological connectivity was established, S-metolachlor was readily mobilised (Stieglitz et al., 2003). Consequently, pesticide export was transport-limited, accounting for 91% of the total seasonal load. As the season progressed and S-metolachlor stores in the catchment were depleted, source dynamics shifted to a mass-limited regime, consistent with previous observations (Peter et al., 2020; Fairbairn et al., 2016).”*

Lines 443-444: This should be made more clear in the methods (Section 2.5.2). Otherwise it is not clear why $\delta^{13}\text{C}$ values need to be predicted for the topsoil, based on a model that quantifies biodegradation in the topsoil already.

This section has now been rewritten (Lines 274 to 279) to address a similar comment from the Reviewer #1. The revised section now reads as follow:

“Therefore, degradation dynamics and extent were inferred from monthly S-metolachlor concentration data (Section 2.3.2) using a degradation model that relates dissipation, degradation, and stable isotope fractionation. This model, previously validated in a nearby agricultural headwater catchment with similar soils and farming practices (Sect. S1.6 in the Supplement; Payraudeau et al., 2025), was applied to independently simulate topsoil S-metolachlor concentrations and corresponding $\delta^{13}\text{C}$ values locations A1 and A2 with higher temporal resolution. Modelled S-metolachlor concentrations were then validated against observed topsoil measurement (Fig. S1 in the Supplement).”

Lines 267-269: Why could be the main reason(s) that this is consistently lower than the 98% mass balance and CSIA-based estimates? Not all TPs accounted for? Or further breakdown (although they are thought to be persistent)? Not enough measurements?

We assume that the targeted lines are Lines 467 to 469. *“Following spring application, a seasonal increase was observed in the proportion of transformation products (sum of ESA, OXA, and NOA) relative to MEL_{SM} (Eq. 1). Mean transformation product proportions were $55.4 \pm 29.9\%$ ($n = 26$) in spring, $84.3 \pm 17.4\%$ ($n = 16$) in summer, $86.4 \pm 9.7\%$ ($n = 22$) in fall, and $95.6 \pm 2.9\%$ ($n = 24$) in winter (Section 2.8: Tab. S12 and Fig. S8 in the Supplement).”*

Considering the upper boundaries of the contribution of three main transformation products, i.e. ESA, OXA and NOA on MEL_{SM}, with 86+9.7% in fall and 95.6 + 2.9% in winter, are not so far to the estimated contribution of degradation close to 98% of the applied S-metolachlor. However, the monthly grab sampling protocol employed by the Water Agency, without corresponding discharge measurements, prevented the accurate integration of TP concentrations into our mass balance calculations.

We have clarified this limitation in relation to the mass-balance analysis in Lines 155–157. *“However, the monthly grab sampling protocol employed by the Water Agency, conducted without simultaneous discharge measurements, precluded accurate integration of TPs concentrations into the mass balance calculations.”*

Figure 4: Why is there this difference in the predicted soil isotope values between A1 and A2? Metolachlor has not been applied in A1 before June? I am not sure whether this has been mentioned before.

The S-metolachlor isotopic signature in topsoil was predicted using the modelling approach developed by Payraudeau et al. 2025 (<https://doi.org/10.5194/hess-29-4179-2025>). Degradation and the associated isotopic signature differ between A1 and A2, reflecting the reported applications and the hydro-climatic dynamics of the topsoil, which explains the differences between the predicted signatures in A1 and A2 topsoil. In the new figure 4, we have averaged the predicted soil isotope values from these two soils and integrated the range of enrichment factor derived from the literature, considering the associated mean and standard deviation ($-1.84 \pm 0.50\text{‰}$ in Table S7).

Accordingly, we have modified the caption (Lines 498 to 505: *“Figure 4: Carbon stable isotope fractionation ($\Delta\delta^{13}\text{C} = \delta^{13}\text{C}(t) - \delta^{13}\text{C}_0$) of S-metolachlor in river ($n= 61$) water across the growing season compared to the predicted topsoil $\delta^{13}\text{C}$ values. The colored dashed lines indicate the median uncertainty in the topsoil $\delta^{13}\text{C}$ values within $\pm 0.5\text{‰}$ (see Table S7 in the Supplement). The shaded light grey area represents the minimal change in isotope signature ($\Delta\delta^{13}\text{C}_{\text{min}}$, calculated as per Eq. S1 in the Supplement) in water, beyond which significant isotope fractionation can be attributed to degradation, thereby representing the threshold for detecting biodegradation. The dark grey segment denotes the estimated date of S-metolachlor application ascertained from the farmer survey within sub-catchment G11. The green line represents the average $\delta^{13}\text{C}$ value from wastewater treatment plant data ($n= 9$). The black dash line represents the $\Delta\delta^{13}\text{C} = 0\text{‰}$ value. Error bars encompass the propagation of uncertainty associated with $\Delta\delta^{13}\text{C}$.”*

Lines 502-503: How are these numbers related to line 384 ("ranging from 0 to 100% of the observed mass load at the catchment outlet")?

We thank the reviewer for this detailed comment and for highlighting the lack of clarity in our statement. The two lines are related: Line 399 specifically refers to the monthly discrete observations at particular time points, whereas Lines 527–528 present the estimates based on mass balance calculations, integrating all sampling days over the season.

In Line 399, we have modified the sentence to specify that it represents the discrete daily load observed on a monthly basis.

“The proportion of WWTP derived S-metolachlor (x_{wwtp}) varied throughout the season (Fig. S6b in the Supplement), ranging from 0 to 100% of discrete daily load observed on a monthly basis at the catchment outlet, with an average contribution of 53% from March to June.”

Lines 527 to 528 have been rewritten for greater clarity as follows: *“Mass balance calculations integrating all sampling days over the season indicate that WWTP effluent contributed between 50% and over 80% of the S-metolachlor load at the catchment outlet.”*

Lines 551-553: I am not sure I understand. Larger catchments with longer in-stream transit times or systems with highly reactive hyporheic zones would lead to more degradation and associated isotope fractionation. Would this not support the applicability of C-CSIA?

We have clarified in lines 577 to 582 that our catchment represents a specific case with limited suitability for applying CSIA to isolate the role of in-stream degradation. We acknowledge, however, that under different hydrological or biogeochemical conditions—such as in larger catchments with longer in-stream transit times—C-CSIA can be highly effective. Lines 577 to 582 have been revised as follows: *“In the Souffel River, a minimum in-stream transit time of approximately 17 days would be required to allow for significant degradation and a measurable $\delta^{13}C$ shift. This constraint illustrates why, in small agricultural catchments with short in-stream transit times and limited hyporheic reactivity, the use of C-CSIA to assess ongoing degradation is limited. In contrast, larger catchments (Strahler order >5) or systems with highly reactive river–sediment interfaces typically exhibit longer in-stream transit times and stronger biogeochemical gradients, which can enhance degradation processes and increase the applicability of C-CSIA.”*

Supplement

- S1.5.2 Estimation of photodegradation in the Souffel river: I cannot follow the calculations easily, as some equations are not fully explained. What is I0? Is there a word missing in “Then, the depth of the photic zone in the Souffel River and the photic zone is defined as follows:”? What are absorbance and intensity used for? They do not reappear in (S4).

We apologise for the lack of clarity in this section. We have carefully reorganized the section and rephrased passages whenever necessary. We believe that Section S1.5.2 in the supplementary Information is now clearer and reads more smoothly.

- S1.6 Variation of S-metolachlor concentrations in topsoil: Please clarify why which parameter is estimated. If I understand correctly, the S-metolachlor degradation is calculated to calculate isotope fractionation with (S9). That's why you get a straight line in Fig. S1. Why don't you use measured S-metolachlor concentrations in (S9)? Because of the limited number of samples?

Topsoil samples (0 to 10 cm) were collected monthly from sugar beet and corn fields at two locations within the surveyed sub-catchment (within a 100 m radius of sites A1 and A2, Fig. 1) to quantify S-metolachlor dissipation over the season. However, isotope fractionation associated with biodegradation in topsoils had to be modeled due to matrix-related analytical issue, using the modelling approach developed by Payraudeau et al. 2025 (<https://doi.org/10.5194/hess-29-4179-2025>), reflecting the reported applications and the hydro-climatic dynamics of the topsoil. This model, validated in a nearby agricultural headwater catchment with comparable soils and farming practices (Sect. S1.6 in the Supplement; Payraudeau et al., 2025), was then applied to predict topsoil S-metolachlor concentrations and the corresponding $\delta^{13}\text{C}$ values at daily temporal resolution for soil locations A1 and A2 independently (Fig. 1). The predicted topsoil S-metolachlor concentrations were validated against the observed concentrations (Fig. S1 in the Supplement).

We have clarified this topsoil modelling step in the revised manuscript (Lines 274 to 279):

“Therefore, degradation dynamics and extent were inferred from monthly S-metolachlor concentration data (Section 2.3.2) using a degradation model that relates dissipation, degradation, and stable isotope fractionation. This model, previously validated in a nearby agricultural headwater catchment with similar soils and farming practices (Sect. S1.6 in the Supplement; Payraudeau et al., 2025), was applied to independently simulate topsoil S-metolachlor concentrations and corresponding $\delta^{13}\text{C}$ values locations A1 and A2 with higher temporal resolution. Modelled S-metolachlor concentrations were then validated against observed topsoil measurement (Fig. S1 in the Supplement).”

S1.9: good to emphasize here (and also in the main text) that this is about in-stream transit times, not transit times through the subsurface

We have replaced *transit time* with *in-stream transit time* throughout Section S1.9 and the main text.

- Caption of Figure S3: “Colours get increasingly red later in the season,”. This is not clear to me –the colour scale has been chosen this way.

The colour scale corresponds to the date and follows the legend presented in the figure. The colour scheme was selected in accordance with the journal’s guidelines and to ensure accessibility for readers with colour vision deficiencies. We carefully evaluated the chosen colours using the Coblis – Colour Blindness Simulator and revised the colour scheme accordingly.

- Table S12: Define that column TPS is indeed transformation products.

TPs and other acronyms have now been clearly defined in the legend of the table S12.