

Reviewer: Violaine Ponsin

This study investigates the degradation of the widely applied herbicide S-metolachlor at the catchment scale through a nine-month field campaign. Two complementary approaches were employed: a conventional mass balance method based on extensive water and soil sampling and concentration measurements, and compound-specific isotope analysis (CSIA). Both methods gave consistent results, indicating that approximately 98% of the applied S-metolachlor is degraded during the growing season. The degradation occurs predominantly in topsoils, while in-stream degradation is limited, primarily due to short water residence times.

This manuscript provides a significant contribution to the field by showcasing the potential of CSIA in assessing pesticide dissipation under field conditions at the catchment scale, and is well presented. It clearly reflects an extensive effort and presents a large volume of data, although navigating the SI is not always straightforward.

The study is well aligned with the scope of HESS, but several comments must be addressed:

We sincerely thank you for your positive and constructive review. We greatly appreciate the time and effort you invested in carefully evaluating our manuscript, as well as your thoughtful identification of details, inaccuracies, and imprecisions that required clarification.

We have now addressed all your comments and suggestions line by line in the blue text below.

1) Isotope fractionation associated with biodegradation in topsoils had to be modeled due to matrix-related analytical issues (I assumed this refers to coelution or high background signal? It would be valuable to explicitly mention and maybe discuss these limitations, as they are relevant for the broader CSIA community).

- It is unclear where the fractionation factor of -1.4‰ used in the model is coming from, as Droz et al. (2021) reported values of either -1.2 or -1.9‰ . Clarification is needed.

The initial calculation used an isotopic enrichment factor of -1.2‰ , corresponding to oxic agricultural soil conditions in Droz et al 2021 (<https://doi.org/10.1021/acs.est.0c06283>). To account for the range of fractionation factors reported in the literature, including Torrento et al. (2021), as highlighted in the following comment, we have now incorporated these values into a revised model. The relevant references are now listed in the updated Table S7 (Supplement), and the associated uncertainty has been propagated throughout the model.

This revision is illustrated in the new Figure S1 and Figure 4, which present the median enrichment factor derived from the literature, along with its standard deviation ($-1.84 \pm$

0.50‰ in Table S7). The changes have been implemented in both the *Materials and Methods* section (line 268-271) and the *Discussion* section (Fig4. line 500).

- Additionally, Torrento et al. 2021 (<https://doi.org/10.1021/acs.est.1c03981>) reported a fractionation factor for carbon of -2.4 ‰ for S-metolachlor biodegradation in soils. It would strengthen the analysis to include a sensitivity test or alternative model run using this value for comparison.

As outlined in the previous point, the range of reported fractionation values is now explicitly incorporated into the model ($-1.84 \pm 0.50\text{‰}$ in Table S7). This adjustment allows us to account for the sensitivity of the predicted $\delta^{13}\text{C}$ values to this experimental parameter, particularly when comparing model outputs with observed $\delta^{13}\text{C}$ values in the river. In response to this comment, we have now revised several sections of the manuscript, including:

L 268: “A median carbon isotopic enrichment factor for S-metolachlor ($\epsilon_{(bulk,C)} = -1.84 \pm 0.50\text{‰}$) was derived from laboratory-controlled biodegradation experiments conducted under a range of conditions reported in the literature (Table S7 in the Supplement). The contribution of photolysis to $\epsilon_{(bulk,C)}$ was considered negligible, based on findings by Van Breukelen (2007) (Sect. S1.8 in the Supplement).”

L 468-469: “the sensitivity of the CSIA method ($\delta^{13}\text{C} = 1\text{‰}$, <43% degradation).” It was <50% before considering the range of reported fractionation values.

L 476: “At the catchment scale, the extent of biodegradation estimated in October using CSIA ($98 \pm 20\%$, $\bar{x} \pm \text{SD}$) was consistent with the overall biodegradation estimated by the mass balance ($99 \pm 5\%$, $\bar{x} \pm \text{SD}$).” It was $98 \pm 2\%$ before considering the range of reported fractionation values.

We have modified the caption (Lines 499 to 506): “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 ($\Delta\delta^{13}\text{C}_{min}$, 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 $\Delta\delta^{13}\text{C}$.”

And in the Supplement:

Lines 164 to 169:

“Boundary values for biodegradation in soil reported in the literature and defined in this study range from $\varepsilon_{biodeg,C} = -2.6 \pm 1.3\text{‰}$ to $-1.2 \pm 0.4\text{‰}$ (Table S7; (Droz et al., 2021; Torrentó et al., 2021; Alvarez-Zaldívar et al., 2018; Meite, 2018). These values were used to determine the extent of degradation. The $\delta^{13}C_0$ represents the isotopic signature of the commercial product (Table S8) (Alvarez-Zaldívar et al., 2018). The model enables the prediction of topsoil S-metolachlor concentrations and corresponding $\delta^{13}C$ values at a higher temporal resolution than that achieved through monthly measurements.

Line 171: A new table S7: “

“Table S7: Range of isotopic enrichment factors for biodegradation ($\varepsilon_{biodeg,C}$) of S-metolachlor reported in the literature for various soils and experimental conditions.”

Line 176: A adapted figure S1

“Figure S1: Measured and predicted S-metolachlor topsoil concentrations at A1 and A2 (Fig. S2). Colored dashed lines represent the predicted uncertainty of the topsoil $\delta^{13}C$ calculate using the uncertainty of the isotopic enrichment factors provided in Table S7.”

2) Section SI 1.7 mentions that three piezometers were installed toward the end of the sampling campaign. Could the authors clarify the rationale behind this installation? What was the intended purpose, and how were the data used in the context of the study? Aside from a brief mention of groundwater electrical conductivity (P12L331), no groundwater data are presented or discussed.

The piezometer installed near the Avenheimerbach River was intended to clarify the influence of the shallow aquifer on river discharge and S-metolachlor loads. However, due to technical difficulties, installation was delayed until the end of the 2019 sampling campaign.

Given the limited value of the resulting dataset, this component of the sampling strategy has been removed from the revised manuscript (formerly lines 331–333). The corresponding Section S1.7 in the Supplementary Information has also been deleted.

Specific comments

P2L60-61: “tracking pesticide degradation under environmental conditions remains challenging due to limitations in current approaches.” Approaches are described but their limitations are not.

We have now clarified the limitation of the current approaches as follows:

L 60-64: *“Despite substantial efforts to study dissipation processes at the catchment scale, tracking pesticide degradation under environmental conditions remains highly challenging. Conventional approaches, based primarily on pesticide concentrations and the detection of transformation products (TPs), reflect both non-degradative dissipation and degradation across catchment compartments, but provide limited knowledge on the specific pathways and the extent of the degradation.”*

P3L65: “although its application has not previously been employed” please reformulate.

We have reformulated this sentence to place greater emphasis on the novelty of our study, as follows:

L.65 *“In this context, compound-specific isotope analysis (CSIA) offers a promising tool for detecting and quantifying contaminant degradation in the environment (Elsner, 2010). However, its application to pesticide degradation in mid-scale agricultural catchments (from 50 to 500 km²) remains largely unexplored.”*

P3L69-70: enables and facilitates.

This has now been corrected.

P5L126: this approach was employed.

This has now been corrected.

P6L143: “water samples from eight monitoring sites”. It is not clear to me whether these sampling points are those shown in Figure 1 (that shows nine sampling points), or different sampling points.

These eight sampling points were part of an independent dataset collected by the local water agency and are indicated in the figure S8 in the Supplement.

We have now clarified this aspect in the revised manuscript as follows, line L141: *“An additional independent dataset from eight locations (Fig. S7 in the Supplement) provided by the Rhin-Meuse Water Agency was used to support evidence of S-metolachlor degradation. “*

P7L158: electrical conductivity.

The sentence has been corrected.

P11L286: please correct “for the same month was the five time drier” & P11L286-287: for every month, or just for some of them (in this case which ones)?

We agree that the original sentence lacked precision, as the comparison referred to the total discharge over the entire study period. Accordingly, L299-304 have been revised as follows:

“Compared to the previous twenty years (March to October 2000 to 2019; <http://www.hydro.eaufrance.fr>), the total discharge was five times lower with an area-normalized discharge at the outlet (A3) of $0.828 \pm 0.986 \text{ m}^3 \text{ day}^{-1} \text{ ha}^{-1}$ ($\bar{x} \pm \text{SD}$; Fig. 2e). 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 $418 \pm 79 \text{ mm}$ (2000–2019 average). In terms of temperature, it was also the third warmest year, with a mean of $16.3 \text{ }^\circ\text{C}$, compared to the 2000–2019 average of $15.7 \pm 0.7 \text{ }^\circ\text{C}$. These data are based on records from the Météo-France station in Entzheim, located approximately 10 km south of the catchment.”

P11L298-299: Figure S6a doesn't really show that up to 100% of the flow comes from WWTP effluents during low-flow periods. Figure S6b does.

Thank you for this comment. Figure S5a (previously S6a) shows that in July and August, the combined effluents from the three WWTPs were approximately three times greater than the upstream river discharge. At the catchment outlet, river–groundwater interactions further reduce surface flow, allowing WWTP effluents to account for up to 100% of the observed discharge (Fig. S6). The influence of these effluents during low-flow periods was even more pronounced for S-metolachlor loads (Fig. S5b), due to the decline in diffuse upstream inputs approximately three months after the last herbicide applications, while point-source releases persist.

We have therefore revised the text to refer to Fig. S6 (previously S7) instead of Fig. S5a (previously S6a), in order to more accurately emphasize the role of WWTP effluents.

P12, L315-318: *“During low-flow conditions, river–groundwater interactions further reduce surface water discharge, causing wastewater treatment plant (WWTP) effluents to contribute up to 100% of the total flow (Fig. S6 in the Supplement). This effect was even more pronounced for S-metolachlor loads (Fig. S5b in the Supplement), highlighting a sharp decline in diffuse upstream inputs roughly three months after the last herbicide applications, while point-source emissions from the WWTP continued.”*

P17L445-446: " However, due to a minimum carbon mass required for accurate GC-IRMS analysis ... only a subset of dissolved water samples (Fig. 4) was measurable." Does this introduce a bias in the reported isotope values, and is this a limitation of the CSIA approach in general? The lowest concentrations are often expected to exhibit the highest levels of degradation.

Out of context, the original sentence may be misleading. Here, we are referring to the instrumental detection limit for accurate measurements, specifically regarding the minimum amount that must be injected into the GC-IRMS system. This does not pertain to environmental concentrations.

We have therefore rephrased lines 465 to 467 as follows:

“However, accurate GC-IRMS analysis of S-metolachlor in the river water required a minimum amount of 20 ng of carbon per measurement. As a result, low-concentration samples lacking sufficient volume for preconcentration could not be analysed. Consequently, only a subset of in-stream samples (Fig. 4) met the criteria for reliable isotopic measurement.”

With modification in the conclusion section 4.2 (lines 567 to 571):

“A key limitation of C-CSIA for S-metolachlor is the relatively high carbon mass required (i.e., 20 ng in this study) for accurate GC-IRMS measurement. This constraint may typically limit its applicability to scenarios where sufficient residual pesticide remains, generally corresponding to degradation extents below 95%. However, pre-concentration techniques such as solid-phase extraction (Gilevska et al., 2022) enabled reliable $\delta^{13}\text{C}$ measurements in river samples through October, even when degradation exceeded 95%.”

P18L454: The uncertainty associated with the extent of biodegradation estimated by CSIA is high compared to that obtained from the mass balance approach, and, according to the authors, this is due to analytical limitations. This point warrants further development.

We have now clarified the meaning of “analytical limitations” in L477 to L481 as follow:
“The higher uncertainty associated with CSIA primarily stems from analytical challenges, such as detecting subtle isotope shifts near the instrumental detection limit, potential matrix interferences, and propagation of uncertainty inherent to Rayleigh-type modelling. Nevertheless, these limitations are partly counterbalanced by key advantages of CSIA, including lower sampling requirements, reduced data demands, and fewer assumptions relative to the mass balance approach.”

Figure 4: it would be helpful to add the $\Delta\delta^{13}\text{C} = 0\text{‰}$ line.

We thank the reviewer for the suggestion to improve the figure’s readability. In response, we have added a reference line at $\Delta\delta^{13}\text{C} = 0\text{‰}$ to enhance interpretability.

P19L492: "the apportionment of S-metolachlor with an isotopic signature distinct from that of agricultural sources". This contradicts P15L390-395, which state that the most plausible

explanation for the occurrence of S-metolachlor in WWTPs is related to “releases during pesticide preparation ... or sprayer clean-out at farmyards”.

We believe that the two sentences are not contradictory. In the first section (L390–395), we discussed the possible entry pathways of S-metolachlor into the WWTPs, identifying sprayer clean-out as the most plausible source. According to this hypothesis, the isotopic signature entering the WWTP should be close to that derived from the commercial products (–31.8‰) which are used (Table S8 in the Supplement). In the second sentence, we discussed the relatively stable isotopic values in the WWTP effluents (around –2‰ compared to initial signature). This stable isotopic signature in the WWTP effluents is likely associated with degradation processes occurring in the activated sludge of these WWTPs. Therefore, sprayer clean-out at farmyards, the most probable source of S-metolachlor entering the WWTPs, can be consistent with the relatively stable isotopic signature observed in the effluents.

We have rewritten Section L514-518, to clarify this point.

“A likely explanation is partial S-metolachlor degradation within WWTPs (Gerecke et al., 2002). Alternatively, some sources previously identified as plausible entry pathways, such as sprayer clean-out or runoff inputs, may introduce S-metolachlor with isotopic signatures slightly altered from those of the original commercial formulations. Disentangling these contributions will require further targeted investigation, which could support the development of more effective mitigation strategies”

P20L498-499: “This indicates that S-metolachlor biodegradation likely occurred between downstream and upstream regions of the Souffel”. Again, this contradicts earlier discussions in the paper, which state that most of the degradation occurs in topsoils. Moreover, a substantial degree of degradation would be required to produce a measurable and significant shift in isotope values.

We believe that the two statements are not contradictory. In the first (P15, L390–395), we discuss potential entry pathways of S-metolachlor into the WWTPs, identifying sprayer clean-out as the most plausible source. Based on this hypothesis, the isotopic signature entering the WWTP should closely match that of the sprayer sample (–31.8‰). The second paragraph does not contradict this interpretation but rather adds clarification by highlighting the comparatively limited degradation potential in the river relative to soil environments. To emphasise this point and strengthen the logical connection between the two paragraphs, we have revised the second accordingly. Consequently, lines 521 to 525 have been rewritten for clarity as follows: *“The observed variations in $\delta^{13}\text{C}$ between upstream and downstream sites suggest the influence of additional processes superimposed on the dominant topsoil degradation signal. These may include minor in-stream biodegradation, potentially occurring near the detection*

limits of our CSIA method, or inputs from a downstream point source containing S-metolachlor that has already undergone partial degradation.”

P20L519-520: “Currently, datasets characterising isotopic fractionation associated with the key pesticide degradation processes, such as biodegradation, photolysis, and hydrolysis, in WWTPs remain scarce”. While this is generally true for many pesticides and micropollutants, it is less true for S-metolachlor, particularly concerning carbon isotopes. See for example Torrento et al., 2021 for hydrolysis and biodegradation (<https://doi.org/10.1021/acs.est.1c03981>) and Levesque-Vargas et al., 2025 for photodegradation (<https://doi.org/10.1016/j.chemosphere.2024.144010>). Although some processes are specific to WWTPs, others are not, and enrichment factors can probably still be applied beyond their original context.

This section now adopts a broader perspective on the application of Compound-Specific Isotope Analysis (CSIA) for tracking the degradation of organic pollutants. We have slightly reworded the text to reflect this more general approach. Accordingly, lines 544 to 550 have been revised as follows: “Comprehensive datasets on isotopic fractionation of herbicides linked to key degradation processes in the environment, such as biodegradation, photolysis, and hydrolysis, remain scarce. While fractionation values have been reported for some herbicides, including S-metolachlor and atrazine, their applicability beyond the original environmental conditions requires rigorous validation. Therefore, further research into herbicide degradation pathways specific to WWTPs is crucial for improving their effectiveness in mitigating micropollutants from urban sources. Such studies would advance our understanding of pesticide residues transformation within WWTPs and support the development of targeted strategies to reduce pollutant loads in aquatic systems.”

P21L526-527 and L548: Water residence times in this catchment are very short. Would this statement remain valid in a watershed with ponds, where longer residence times are expected?

Our statement that “C-CSIA at the catchment outlet effectively reflects the extent of S-metolachlor degradation in topsoil across scales” remains valid, even for catchments with longer in-stream transit time and more advanced degradation. However, the sampling strategy, particularly the choice of water volumes, and the extraction and concentration steps using solid-phase extraction, should be optimised to enable the detection of more ^{13}C -depleted $\delta^{13}\text{C}$ values in water, which are indicative of lower residual S-metolachlor concentrations.”

Accordingly, lines 578 to 583 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 $\delta^{13}\text{C}$ 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.”

Supporting information

The table of contents should be more detailed to facilitate navigation.

The tables have now been more detailed to facilitate navigation.

The Y-axis unit should be revisited in Figure S1.

We have now revised the unit of the S-metolachlor concentration as µg per kg of soil.

Section S1.9: The text in this section suggests that fractionation factors for two different elements, C and N, were used in Equation S13 to derive a single effective fractionation factor (P10L191-192). This point requires clarification.

Thank you for this comment. The wording in Section S1.9 was indeed inaccurate and unintentionally implied that carbon and nitrogen fractionation factors were combined into a single effective factor in Equation S13. This was an oversight on our part. We have now corrected the section to clearly describe the distinct and intended application of the individual carbon and nitrogen fractionation factors.

We have removed the mention of nitrogen fractionation factors in the sentence (Lines 215-217):

“Contrasted isotopic enrichment factors as been observed for indirect photodegradation ($\epsilon_{photo,C} = 0.0 \pm 0.0\text{‰}$ in Drouin et al. (2021) and $-0.4 \pm 0.1\text{‰}$ in Levesque-Vargas et al. (2025)) and for biodegradation in soil ranging from $\epsilon_{biodeg,C} = -2.6 \pm 1.3\text{‰}$ to $-1.2 \pm 0.4\text{‰}$ (Droz et al., 2021; Torrentó et al., 2021; Alvarez-Zaldívar et al., 2018; Meite, 2018).”

P15L281-282: Levesque-Vargas et al., 2025 reported isotopic enrichment factors for S-metolachlor photodegradation, although it was limited ($-0.4 \pm 0.1\text{‰}$ during indirect photodegradation).”

We thank the reviewer for highlighting the variability in isotopic fractionation values reported by different authors. We have evaluated both values in our calculation (see SI L216 to 225) and found that the range of isotopic enrichment factors reported in the literature for metolachlor photodegradation does not significantly affect the effective

isotopic fractionation (ϵ_{eff}) when using the Van Breukelen method (<https://doi.org/10.1021/es0628452>) which account for the different kinetic and isotopic enrichment factor when multiple degradation pathway occur.

We have now clarified this issue in the main text L263

“The contribution of the photolysis on the $\epsilon_{bulk,C}$ has been estimated as negligible for S-metolachlor following Van Breukelen (2007) (Sect. S1.8 in the Supplement).”

as well as in the SI L219 to 225.

“As photodegradation and biodegradation co-occur in rivers, the Rayleigh Eq. (S9) was corrected according to Van Breukelen (2007) Eq. (S13):

$$\epsilon_{eff,c} = \frac{k_{photo} \times \epsilon_{photo,C} + k_{biodeg} \times \epsilon_{biodeg,C}}{k_{photo} + k_{biodeg,C}} \approx \epsilon_{biodeg,C} \quad (S13)$$

with ϵ_{eff} the effective isotopic enrichment factor to be re-injected in Eq. (6) to define the extent of in-stream degradation. *However, the photodegradation term in Eq. S13 is negligible; consequently, in the present case study, $\epsilon_{eff,c}$ can be considered equivalent to $\epsilon_{biodeg,C}$.*”

Figure S6, X axis: it should be “sept” instead of “oct”.

Thank you for identifying this; it has been corrected.