Author's response

Living cover crops reduce pesticide residues in agricultural soil

Reviewer comments are integrated in red. Our responses and proposed changes are included in black. Line numbers refer to the initial manuscript.

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1) Response to RC1 (Eglantina Lopez Echartea)

a) General comments

This manuscript presents an investigation into the influence of cover crops on the fate of multiple pesticides compounds in soil and soil solution under pots and greenhouse conditions. The experimental design is innovative in applying a broad-spectrum pesticide mixture and monitoring its dynamics over time using analytical techniques. The PCA and physicochemical profiling contribute to a better understanding of how chemical properties influence persistence and distribution. However, the study makes several claims, particularly around microbial degradation and rhizosphere effects that are not fully supported by direct measurements. While the authors acknowledge some limitations, further caution is required in the interpretation of mechanisms. Overall, the manuscript provides valuable data but needs significant revisions in terms of how conclusions are framed.

Thank you for your thoughtful and constructive comments. Please find below our detailed responses. As it was requested that a revised manuscript should not be submitted with our response, the proposed changes are described with reference to line numbers from the preprint version of the manuscript.

b) Specific comments

i) Scientific significance

The study offers useful insight into pesticide-soil interactions and the potential of cover crops to affect pesticide retention and degradation. However, its broader contribution is limited by the absence of key complementary measurements (e.g., microbial activity, metabolite formation, evapotranspiration, accumulation in plants). These gaps limit the mechanistic depth needed to substantially advance the field.

We appreciate your emphasis on the importance of complementary measurements to deepen the mechanistic understanding of pesticide–soil interactions.

As stated in lines 92–94, we hypothesised based on existing literature that pesticide accumulation in plants over an 80-day period plays a lesser role than soil degradation, justifying the exclusion of plant tissue analysis in this study. We propose to reclarify this in line 466 by adding: "*since plant uptake generally plays a smaller role in pesticide dissipation than soil degradation (Tarla et al., 2020)".* While we recognise that this limits the mechanistic depth of our findings (as acknowledged in lines 525–526), this decision was also influenced by practical constraints, including limited laboratory capacity to analyse an additional matrix and financial limitations on the number of analyses that could be performed.

The same applies to microbial activity monitoring (explicitly acknowledged as a primary limitation in lines 482–484 and 512–513) and metabolite quantification (mentioned in line 155 and further discussed in lines 486–494 and 515).

We hope that the preliminary results presented in our paper will help to support future research efforts and funding aimed at broadening the analytical scope. Ideally, this would include the quantification of a wider range of active substances and their metabolites, the monitoring of plant uptake and the microbial dynamics, and the use of an experimental set-up that takes into account for both leaching and evapotranspiration processes.

ii) Interpretation of results

- Several conclusions suggest microbial degradation or rhizospheric effects (e.g., Fig. 4 and the discussion around "efficient degradation"), yet no microbial, enzymatic, or metabolite data are provided. These should be clearly framed as hypotheses rather than findings.
- The conclusion that increased pesticide content under thin cover crops reflects rhizofiltration (rather than slower degradation or less mobility) is speculative without additional data on leaching or degradation pathways.

To further clarify that microbiological monitoring was not included in the analysis, we propose to revise lines 94–96 into: "*As our objective was to identify trends in the physicochemical properties*

of the active substances affected by cover crops, we did not include microbiological monitoring in our analysis."

To mark a clearer distinction between our results and the hypothesised mechanism, we propose in section 3.2 to move the results and discussion on the behaviour of the three contrasted molecules (lines 312–321, 339–347, and 348–357, as well as Figure 3) to the Supplement and to clearly rename the section into "*Hypothesised mechanism*". See also our response to your comment on Figure 4 and the conceptual model.

To accommodate these modifications, we propose to modify lines 306–311 into: "*The shifts analysed in the previous section highlight the dynamic speciation and redistribution of compounds within each soil compartment over time. PERMANOVA results showed that, after soil compartments and sampling dates, cover modalities were the third most statistically significant factor explaining the variability in pesticide content between samples. Focusing on soil samples, the evolution of pesticide content over time and between cover modalities —detailed in Supplements S3 and S4— showed a dual trend after 80 days: (1) higher retention under thin cover (relative to thick cover and control), and (2) greater reduction under thick cover (relative to thin cover and control). These patterns support our two main hypotheses: (1) that rhizofiltration, driven by evapotranspiration, contributes to pesticide retention under less developed covers, and (2) that enhanced microbial biodegradation under thicker, more developed covers drives pesticide degradation. This leads to the following hypothesised mechanism:*". See also our response to your comment on the statistical testing.

iii) Soil and system properties

The study does not monitor soil physicochemical parameters over time (e.g., pH, organic matter, nutrients, microbial biomass), which are known to influence pesticide dynamics and could be differentially affected by the cover modalities. Their omission limits confidence in treatment effect attribution.

We agree that soil physicochemical parameters —such as pH, organic matter, nutrient content, and microbial biomass— can influence pesticide dynamics and may themselves be influenced by cover crops. Differential changes in these parameters could contribute to the observed differences in pesticide dynamics between modalities, and their absence limits the ability to confidently attribute observed effects to specific mechanisms.

However, all modalities were conducted on the same homogenised soil, and the 80-day period of cover crop growth is unlikely to be sufficient for significant divergence in bulk soil properties. While cover crops are known to affect these parameters over time —particularly after termination with incorporation, which is outside the scope of our study—, existing literature suggests that significant changes in bulk soil properties typically require several years of cover cropping (Blanco-Canqui et al., 2023; Wang et al., 2020). We propose to precise this by adding the following after line 153: "*As all modalities were conducted on the same homogenised soil, and given that significant changes in bulk soil properties generally require several years of cover cropping (Blanco-Canqui et al., 2023; Wang et al., 2020), we considered the 80-day cover crop growth period insufficient to induce meaningful divergence in soil physicochemical parameters (e.g. pH, organic matter, nutrients). Consequently, these parameters were not monitored beyond the initial soil characterisation.*"

We acknowledge that localised rhizosphere scale effects do occur and that their monitoring could help increase the confidence in the proposed hypothesised mechanism. However, investigating such micro-scale mechanisms was beyond our capabilities at the time of the experiment.

We agree that future studies incorporating temporal monitoring of soil properties and microbial dynamics would be valuable to better disentangle the mechanisms underlying observed effects, as noted in lines 503–505 and 521–523 of the manuscript.

iv) Statistical testing

The PCA in Figure 2 provides an summary of variance but lacks statistical support. Consider adding a PERMANOVA or similar test to assess whether observed groupings (e.g., by compartment or time) are statistically significant.

We appreciate this insightful suggestion and have addressed it by conducting a PERMANOVA to statistically support the groupings observed in the PCA. We propose the following modifications to the MS:

- Addition at the end of line 194: "*Permutational multivariate analyses of variance (PERMANOVA) were performed on the PCA to discuss results, using the* R *function* `vegan::adonis2` *(Oksanen et al., 2025). The homogeneity of the multivariate dispersion between the analysed groups was confirmed (*p-*value > 0.52), supporting the robustness of the observed patterns.*"
- Addition after line 304: "*These visual patterns were statistically supported by PERMANOVA, which demonstrated that soil compartment, sampling date, and cover modality each independently and significantly influenced the distribution of active substance levels. Compartment alone accounted for 68.5 % of the variance (*p-*value < 0.001), while date and modality explained 19.4 % (*p-*value < 0.001) and 16.0 % (*p-*value < 0.01), respectively. Combined, these three factors explained 88.3 % of the variance, increasing to 91.5 % when interactions were included. These results confirm that the separation observed in PCA space reflects differentiated trajectories of active substance evolution across soil compartments, sampling times and cover modalities.*"

See also the proposed change to the next paragraph (lines 306-311) in our response to your comment on interpretation of results.

v) Relevance of application rates

Please clarify how the pesticide application compares to realistic field conditions. Are the dosages representative of actual agricultural use, or are they higher for analytical purposes?

As stated in line 113, we applied pesticides at their maximum authorised dose, as there are no official 'standard doses' for pesticide use in Belgium. This may in some cases overestimate application rates compared to typical field practices. However, we used soil from a certified organic plot (see line 107) to minimise background pesticide contamination. This choice may in turn lead to an underestimation of residue levels found in conventional soils. We believe that the combination of these potential over- and underestimations results in pesticide residue levels that are representative of average field conditions. This interpretation is supported by comparison with findings from other studies in the literature (see lines 255–265 and 283–291).

vi) Metabolite monitoring

The lack of metabolite analysis is a critical limitation. Many pesticide degradation products can be more toxic or persistent than their parent compounds. This should be discussed in more detail and noted explicitly in the conclusions.

We acknowledge that the lack of metabolite quantification is a critical limitation of our study, as you pointed out. This issue is raised in line 155 and discussed in more detail in lines 486–494 and 515, particularly in relation to the potentially greater toxicity or persistence of some metabolites compared to their parent compounds.

At the time of the study, we faced analytical and financial constraints that limited our ability to systematically include metabolites in the analysis. Given these constraints, we chose to focus on a broad range of active substances to capture a variety of physicochemical behaviours, rather than conducting a more targeted and comprehensive tracking of metabolites of each substance. While this approach prevents us from drawing conclusions about degradation pathways, it does not affect the observed patterns of pesticide distribution and retention, nor the established thresholds of physicochemical properties of degraded active substances and cover densities to achieve significant reductions in pesticide levels.

In response to your suggestion, we propose to make this limitation explicit in the conclusion by adding the following sentence at the end of line 548: "*Major limitations of this study include the lack of direct measurements of soil microbial biomass and activity, and the lack of systematic assessment of pesticide metabolites*."

vii) Figure 4 and conceptual model

The caption and narrative accompanying Figure 4 suggest definitive degradation by microbiota, which is not demonstrated in the study. This should be revised to reflect a proposed mechanism, unless additional data are included.

Thank you for pointing out the need to clearly distinguish between our observed results and the hypothesised mechanism. To address this, we propose the following revisions:

- **Restructuring Section 3.2**: We propose to move the results and discussion regarding the behaviour of the three contrasted molecules (lines 312–321, 339–347, and 348–357, as well as Figure 3) to the Supplement and to retitle the section "*Hypothesised mechanism*." This will clearly separate Section 3.2 from the observed results and allow it to focus exclusively on the proposed hypothesised interpretation, thereby reinforcing the distinction between empirical findings and mechanistic speculation. See also our response to your comment on result interpretation.
- **Clarifying the narrative:** To further emphasize the putative nature of the mechanism, we propose to adapt its description in various places —e.g. "As the cover develops, *we hypothesise that* the thin cover modifies soil water fluxes" in line 322, "The higher content under the thin cover crop *would* therefore reflect a greater retention" in line 324, "suggesting that it *would* depend not only on the stage of development of the cover" in line 328, etc.— and to insert the following sentence at the end of line 331: *"As evapotranspiration, leaching, microbial activity and metabolites were not analysed, we cannot confirm this hypothesised mechanism."*

- **Updating the figure caption:** We propose to revise the caption of Figure 4 to read: *"Hypothesised mechanism: cover crops reduce pesticide leaching by modifying soil water fluxes through evapotranspiration, thereby concentrating pesticides in the rhizosphere, where they may be more effectively degraded by soil microbiota."*

These modifications lead to small revisions in lines 366–368, in the caption of Figure 5 and in lines 493 and 544.

In addition, we propose to remove lines 422–423.

viii) Water management and potential runoff

The manuscript does not describe whether the pots were equipped to prevent water runoff or collect leachate, which is particularly relevant given the study's focus on pesticide fate. While soil moisture was monitored and controlled through watering regimes, it is unclear whether pots had drainage holes or whether leached water was collected or measured. If drainage occurred and was not accounted for, this could confound interpretations of pesticide disappearance as degradation versus loss. Please clarify how water balance and potential runoff or leaching were managed throughout the experiment.

Thank you for this important observation. We acknowledge that this detail was missing from the preprint. To address your comment, we propose to add the following clarification at the end of line 131: "*To prevent water runoff and uncontrolled leaching, each pot was placed in an individual saucer with sufficient capacity to retain any excess irrigation water. Saucers were monitored after each watering throughout the experiment, and no overflow was observed, confirming that all drainage water was fully retained.*"

We agree that this design, combined with the limited depth of the pots, limits the extrapolation of our results to field conditions, where vertical drainage towards deeper soil horizons occurs regularly (as discussed in lines 495–503). To explicitly acknowledge this limitation, we also propose to add the following sentence at the end of line 519: "*, and sample soil and soil solution at different depths to better assess the vertical mobility of pesticide residues;"*

c) Technical corrections

- Line 295: Replace "witch" with "which."
  Thank you for your careful review. This typographical error has been corrected as suggested.
- Lines 73 and 79: Citations to Pelletier and Agnan are missing the publication year.
  The missing publication years have been added for Alletto et al. (line 68) and for Pelletier and Agnan (lines 73 and 79), as recommended.
- Line 80: The statement about bioremediation prioritizing reactors over field applications needs citation. Consider rephrasing or supporting with evidence, as it oversimplifies the field's current practices.
  We propose to rephrase lines 80–84 as follows: "*Despite progress in the literature, two main limitations remain: (1) field research is often limited to a narrow range of pesticide molecules and cover crops, with inconsistent assessments of soil compartments; and (2) influence of cover crops is generally not analysed in relation to the physicochemical properties of the molecules. These gaps prevent a broader understanding of the general*

applicability of cover crop remediation strategies for different pesticide molecules." and to remove line 87 in consequence.

d) Conclusion

We acknowledge the limitations of our study and hope that the preliminary results presented will support future research efforts and funding aimed at expanding the analytical scope. Ideally, such efforts would include the quantification of a wider range of compounds and their metabolites, the monitoring of plant uptake and microbial dynamics, and the use of an experimental design that accounts for both leaching and evapotranspiration processes.

We trust that the proposed revisions will help to clarify the exploratory nature of the hypothesised mechanism, while highlighting its potential as a basis for future investigations.

References

Blanco-Canqui, H., Ruis, S. J., Koehler-Cole, K., Elmore, R. W., Francis, C. A., Shapiro, C. A., Proctor, C. A., & Ferguson, R. B. (2023). Cover crops and soil health in rainfed and irrigated corn: What did we learn after 8 years? *Soil Science Society of America Journal*, *87*(5), 1174–1190. https://doi.org/10.1002/saj2.20566

Oksanen, J., Simpson, G. L., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Solymos, P., Stevens, M. H. H., Szoecs, E., Wagner, H., Barbour, M., Bedward, M., Bolker, B., Borcard, D., Carvalho, G., Chirico, M., Caceres, M. D., Durand, S., … Borman, T. (2025). *vegan: Community Ecology Package* (Version 2.6-10) [Dataset]. https://cran.r-project.org/package=vegan

Wang, Y., Liu, L., Tian, Y., Wu, X., Yang, J., Luo, Y., Li, H., Awasthi, M. K., & Zhao, Z. (2020). Temporal and spatial variation of soil microorganisms and nutrient under white clover cover. *Soil and Tillage Research*, *202*, 104666. https://doi.org/10.1016/j.still.2020.104666

2) Response to RC2 (anonymous)

Vandevoorde et al. monitor pesticide residue levels in soil and soil solution under two different cover crop densities. The results showed that, compared to bare soil, thin cover crops reduce pesticide leaching after sowing for 80 days. In addition, well-developed cover crops reduce soil pesticide contents by more than 33%. The experimental design is clear, and the results help to reduce pesticides in soil. I have several suggestions that may improve the MS quality.

a) Comment 1

The novelty of the manuscript is not clearly established; for example, including more pesticides in the study should not be regarded as an innovation.

We appreciate this comment and agree that simply increasing the number of pesticides tested is not in itself a novel contribution. The core novelty of our study lies in addressing a specific gap in the literature: while most previous research has focused on the effects of *established* cover crops or mulches on *newly applied* pesticides (see lines 56–58), our study investigates how *newly sown*

living cover crops influence the fate of *pre-existing* pesticide residues. This distinction is critical for evaluating the phytoremediation potential of cover crops within active cropping systems (without taking land out of production) and for distinguishing our focus from the more common evaluation of the effects of cover crops on freshly applied pesticides.

The inclusion of multiple pesticide molecules in our study is not an innovation, but a methodological necessity to assess whether the fate of pesticides under cover crops could be predicted based on the physicochemical properties of the active substances and the density of the cover crop. This required a wide range in the values of the physicochemical properties, which required a wide range of selected active substances.

To make this contribution explicit in the manuscript, we propose to revise lines 80–84 on the literature gap as follows: "*Despite progress in the literature, two main limitations remain: (1) field research is often limited to a narrow range of pesticide molecules and cover crops, with inconsistent assessments of soil compartments; and (2) influence of cover crops is generally not analysed in relation to the physicochemical properties of the molecules. These gaps prevent a broader understanding of the general applicability of cover crop remediation strategies for different pesticide molecules.*"

b) Comment 2

The key issue is the lack of explanations for the results, e.g., microbial degradation is not involved in the study.

We fully agree that mechanistic processes such as microbial degradation were not directly measured in our study, and we thank the reviewer for pointing out the need to clearly distinguish between observed data and proposed interpretations.

To reinforce this distinction, we propose the following revisions:

- **Restructuring Section 3.2**: We propose to move the results and discussion regarding the behaviour of the three contrasted molecules (lines 312–321, 339–347, and 348–357, as well as Figure 3) to the Supplement and to retitle the section "*Hypothesised mechanism*." This will clearly separate Section 3.2 from the observed results and allow it to focus exclusively on the proposed interpretation, thereby reinforcing the distinction between empirical findings and mechanistic speculation. See also our response to your comment on result interpretation.
- **Clarifying the narrative:** To further emphasize the putative nature of the mechanism, we propose to adapt its description in various places and to insert the following sentence at the end of line 331: *"As evapotranspiration, leaching, microbial activity and metabolites were not analysed, we cannot confirm this hypothesised mechanism."*
- **Updating the figure 4 caption:** We propose to revise the caption of Figure 4 to read: *"Hypothesised mechanism: cover crops reduce pesticide leaching by modifying soil water fluxes through evapotranspiration, thereby concentrating pesticides in the rhizosphere, where they may be more effectively degraded by soil microbiota."*
- **General adaptations throughout the manuscript**: We will also refine the wording in several locations to make a clearer distinction between observed results and proposed mechanisms.

c) Comment 3

Please carefully check the overall writing of the MS, making it more concise, rigorous, and reliable. e.g., lines 530-535 are not conclusions.

We have reviewed the manuscript with the aim of improving the conciseness, rigour, and precision of the narrative. We propose several cuts and refinements throughout the manuscript to streamline the text and focus on key messages —for example, in lines 30–34, 81–82, 87, 312–321, 339–347, 349–357, 423, 437–445, 517–518 and 530–533, as well as the removal of Figure 3. Specifically, we propose to move lines 312–321, 339–347, and 348–357, along with Figure 3, to the Supplement (see our response to RC1 for details).

In response to your specific point regarding lines 530–533, we agree and propose transfer them to the discussion in line 453.

In order to maintain rigor and reliability, we have retained the Materials and Methods and the core of the Results and Discussion sections intact. Additional improvements to the interpretation and discussion —particularly in distinguishing data from hypotheses— are addressed in our response to you second comment above.