Responses to the 2nd reviewer's comments on the preprint

"Diachronic assessment of soil organic C and N dynamics under long-term no-till *cropping systems in the tropical upland of Cambodia"*

1. Reviewer's Expression & General comments

Comment: *This study explored the soil organic C and total N dynamics in the soil profile of 0-100 cm under long-term no-till cropping systems (conventional tillage, NT monocropping, and NT crop rotation systems) in the tropical upland of Cambodia using a diachronic and equivalent soil mass approach. The results of this study showed that adopting NT cropping systems with diverse crop and cover crop species increased SOC accumulation (SOC concentration, SOC stocks) in the whole depth, by increasing both the C pools in the POM and MAOM size fractions but raised questions about soil N dynamics (TN concentration, TN stocks). However, there are still some important issues about study objectives, result analysis and discussion that need to be addressed before being accepted. Detailed comments are as below.*

Response: We really appreciate the time and effort that you have put into offering feedback and comments on our preprint. We are grateful for your constructive remarks that help us to improve our article. We have considered all your comments and suggestions in our extensive responses.

Comment: *1. This study mainly aimed to quantify the impacts of CT and different NT cropping systems on SOC and TN dynamics, thus the comparison between diachronic approach and synchronic approach should not be included in the scientific hypotheses. Note that the C pools in the POM and MAOM size fractions should be included in the hypotheses. Suggest moving the results of method comparison to supplementary materials.*

Response: Thank you very much for your suggestions. We agreed to remove the comparison of the diachronic approach and the synchronic approach from the hypotheses as this was not the main objective of this paper. This sentence "*In addition, calculating SOC stock using the diachronic approach would prevent a biased estimation of the SOC accumulation when compared to the synchronic approach*" has now been deleted. However, we think that it is important to keep this result in the discussion rather than moving it to the supplementary materials because our findings, in alignment with others, suggest that the synchronic approach, despite its simplicity and lower requirements of time and resources, can potentially result in incomplete or misleading conclusions. We think this comparison largely improves our discussion, so we prefer to keep it there.

Concerning the C pools in the POM and MAOM size fractions, we agree with you. We have now included it in the hypotheses by slightly modifying the current sentence: "*We hypothesized that implementation of the three core technical principles of CA would significantly enhance the SOC stocks, both in the POM and MAOM size fractions, including in the subsoils.*"

Comment: *2. Multiple crop species were included in these NT cropping systems, which will significantly affect the accumulation of soil organic carbon and total nitrogen. However, the potential mechanisms were not discussed or explained in this study.*

Response: Thank you very much for raising this important point. To describe the roles and mechanisms of crop diversification in the accumulation of SOC and TN, the paragraph below was inserted in between L132 and L133 of the introduction section: "*Soil organic C sequestration is closely related to soil aggregate structure (Six et al., 2004; Liu et al., 2021). The complexity of cropping systems, characterized by crop species diversity through the use of cover crops, crop rotation, and intercropping, was reported to enhance soil aggregation stability and proportion of soil macroaggregates, along with the increase of soil organic carbon (Tiemann et al., 2015; Li et al., 2024; Poeplau et al., 2024). The diversity of crop species increased the quality, quantity, and chemical diversity of plant-derived litter inputs, which are the main sources of energy for soil microorganisms, and increased microbial activity and the abundance of fungal and bacterial communities (Tiemann et al., 2015; Zhang et al., 2023). The overall increase in fungal hyphae, plant roots, and aboveground biomass inputs under crop diversification are important organic binding agents that promote the formation of macroaggregates and facilitate the soil aggregation process (Tiemann et al., 2015). Furthermore, the increased amount and diversity of plant-derived C inputs in the forms of crop residues and root exudates provided a suitable microenvironment for soil microorganisms, which promoted microbial growth and turnover (Morugán-Coronado, 2022). The faster microbial growth and turnover rates increased the amount of microbial biomass and necromass, thus increasing SOC (Liang et al., 2011; Prommer et al., 2019).*"

Comment: *3. What are the advantages and disadvantages of the diachronic and synchronic approaches? The background should be described in the Introduction, as this study emphasizes that the diachronic method can prevent biased estimation of SOC accumulation compared to the synchronous method.*

Response: Thank you very much for pointing this out. To emphasize the importance of using the diachronic approach for our study, we modified the paragraph from L145-149, and replaced by the following paragraph to the main text: "*In addition, using improper methods could mislead the assessment of the long-term*

impact of management practices on the SOC stock. There are two different soil sampling approaches for assessing SOC accumulation rates: the diachronic and the synchronic approaches (Bernoux et al., 2005). The diachronic approach refers to collecting samples on the same field plots over time. The synchronic approach, also known as the space-for-time method, on the other hand, refers to sample collection at the same time from different (often adjacent) field plots under different land-use or management systems (Bernoux et al., 2005; Neto et al., 2010). Neto et al., (2010) and Junior et al., (2013) revealed that the synchronic approach led to overestimated SOC accumulation from long-term experiments in Brazil due to spatial heterogeneity and initial land use history. They highlighted that diachronic soil sampling should be used for assessing soil SOC storage rates due to changes in land-use or management patterns because it offers a more comprehensive view of how SOC and N levels change under long-term tillage and cropping systems over time in which non-identical initial soil conditions cannot practically be excluded, making it more accurate and realistic for the investigation of SOC and N dynamics, despite the fact that they are costly and require significant time and resources (Bernoux et al., 2005; Neto et al., 2010; Junior et al., 2013). The synchronic approach, on the other hand, is simpler, lower-cost, and less time-consuming, but they may overlook the effects of NT systems over time since it is impossible to eliminate all environmental factors other than the impacts of NT systems that influence SOC and N content because of the high spatial variability of land use history prior to the conduct of the experiments (Neto et al., 2010; Junior et al., 2013).”

Comment: *4. Unfortunately, this paper reads more like a research report than a scientific article. It is confusing to understand the important results when SOC and total N (TN) are described together without a clear logical order. New subheadings are recommended to distinguish the results for SOC and TN. In addition, it would be better to combine the results for maize, soya and cassava cropping systems into same section rather than using the subheading for each system.*

Response: Thank you very much for your pointing this out, and we appreciate your insightful suggestion. We modified the result sections complying with your recommendation and recommendations from Reviewer 1 by merging the results of maize, soybean, and cassava together with logical orders and hierarchy of importance (from most importance to unexpected findings) with two separate subheadings for SOC and TN.

Comment: *Rather than detailing the results of published papers (e.g. L602-630, 631-640, etc.) and replicating the results of this study (e.g. L623-630, etc.), it is more important to effectively discuss the mechanisms by which different crop rotation systems under NT affect SOC and TN in this study.*

Response: Thank you very much for the suggestion. However, we think that it is critical to first compare our findings on SOC stock change to other studies in tropical and other climatic regions, which we did from L602 to L640 in the discussion section. Then, we extensively discuss the potential mechanisms involved, beginning with L641 to L699 in the discussion section.

2. Reviewer's specific comments

Comment: *Line 31-66. The abstract is generally limited to 400 words or less, please condense the text.*

Response: Thank you for the recommendation. There were no specific guidelines of the journal about the length of the abstract, but we tried to shorten it, from 598 words to 429 words.

Comment: *Line 42-58. The results of the SOC and TN dynamic changes under CT should be described in the abstract.*

Response: Thank you very much for your suggestion. We had incorporated the results of SOC and N dynamics under CT into the new version of the abstract.

Comment: *Line 42-58. Since the main results showed the differences under diverse cropping systems during the 10-year period (2011-2021), a clear comparative year (2011 and 2021) should be added at the beginning of these sentences.*

Response: Thank you very much for pointing this out. We included the clear comparative year at the beginning of result description in the new version of the abstract.

Comment: *Line 42-44. P value > 0.05? if the significant difference was observed, the P value was less than 0.05. Please check and revise.*

Response: We apologies for this typo error. We corrected it.

Comment: *Line 81-98, 153-168. Suggest integrating these two paragraphs.*

Response: We understand the reviewer's suggestion merging the two paragraphs together. This is the same suggestion as the first reviewer. Taking into account the two suggestions, we have split the paragraph (L153–168) into a few parts, reshaped some phrases, and merged each of them into another paragraph of the introduction as follow: "*Cambodian soils are seriously threatened by intensive agricultural systems. The returns on taking actions against land degradation are estimated at 3 US dollars for every dollar invested in restoring degraded land in*

Cambodia (UNCCD, 2018). Therefore, taking actions to reverse the trend of soil degradation through restoration and adopting sustainable agricultural management practices highlights the strong economic benefits of combating soil degradation in the country (UNCCD, 2018)" into L98.

The phrase "*Since 2004, CA research for development program has been initiated in Cambodia by the joint collaboration between the General Directorate of Agriculture (GDA) and the Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), France*" was incorporated into L112.

The phrase "*CA have been promoted to smallholders in various agroecosystems of Cambodia since 2009. The early effects of CA cropping systems on soil health and SOC sequestration have been reported in several studies (Hok et al., 2015, 2018, 2021; Pheap et al., 2019; Suong et al., 2019; Sar, 2021; Koun et al., 2023); however, information on the impact of long-term CA systems on the changes in SOC stock remains scarce in the country as well as in Southeast Asia. There is a need to document the long-term changes in SOC stock under CA cropping systems to fill in the knowledge gaps as well as provide robust evidence to land use planners and policymakers. This could be profitable not only for Cambodia but also for the whole region*" was merged to the main text in L153.

Comment: *Line 170-174. Add the abbreviations (TN) for total N stocks, please check and revise it in the main text.*

Response: Thank you very much. We have added the abbreviation "TN" for total N stocks to the entire main text of the manuscript.

Comment: *Line 183. Please check and add the SOC content or SOC stocks.*

Response: Thank you very much for pointing this out. We have included the SOC stocks of the pre-experiments into the sentence.

Comment: *Line 190. In section 2.2, the planting date and harvest date of multiple crops should be added under different cropping systems.*

Response: Thank you very much for bringing this out attention. After L207, we included the following new paragraph to the main text: "*Under the CA systems, the species, dates, and methods of cover crop establishment varied depending on the design of treatments for each experiment, the types and cycles of the main crops, and the species and cycles of the cover crops (Table 1 in the supplementary materials). For instance, Stylosanthes guianensis and Brachiaria ruziziensis were associated with rice and soybean, respectively, by manual broadcasting at the full flowering stage of rice before the end of September and at the first yellow*

leaves of soybean in the mid of October. Stylo was associated by line sowing with a NT planter at the same date of maize cultivation and 20 days after planting for cassava. In addition, if the development and/or density of the cover crop sown the previous year were considered insufficient, short cycle cover crop species, i.e., pearl millet or sorghum, was sown alone or mixed with cowpea and sunnhemp at the beginning of the rainy season (in the first week of May). Cover crops were then grown for 60–75 days to increase the biomass inputs prior to the cultivation of the main cycle of rice, soybean, or maize (Table 1 in the supplementary materials)."

In addition, before L208, we added the following paragraph: "*The establishment and harvest of the main crops varied depending on the species. For maize, upland rice, and soybean, with a life cycle of approximately 110–120 days, these crops were mainly seeded between the last week of June to mid-July and harvested between mid-October and mid-November, whereas cassava was planted in early May and harvested around 10 months old in the mid-February of the following year*."

Comment: *Line 203-207. Please separately describe the treatment iii and iv.*

Response: Thank you very much. Consolidating the first reviewer' comment and your suggestion on the treatment description (L 199-207), we modified the names and split the description of treatments iii and iv, and replaced it with the following phrase: "*Each experiment consists of four (4) treatments. Treatment (1): monocropping under conventional tillage (CTM), in which the main crops, i.e., maize (Mz), soybean (Sb), and cassava (Cs), are monocropped with land preparation done by disc ploughing (CTM-Mz, CTM-Sb, and CTM-Cs). Treatment (2): monocropping under conservation agriculture (CAM), in which the main crops (maize, soybean, and cassava) are cropped in a one-year frequency pattern under CA management (CAM-Mz, CAM-Sb, and CAM-Cs) with no soil tillage along with the addition of cover crops. Treatments (3) and (4) were the bi-annual crop rotation systems of the main crops under CA management (CAR1 and CAR2) with no soil tillage along with the use of cover crops. For treatment (3) of SoyEx and CasEx, represented by CAR1-Sb and CAR1-Cs, respectively, the main crops (i.e., soybean and cassava) were grown in a bi-annual rotation with maize in treatment (4), represented by CAR2-Sb and CAR2-Cs for SoyEx and CasEx, respectively. For the treatment (3) of Mai-Ex, the main crop (i.e., maize represented by CAR1-Mz) was grown in a bi-annual crop rotation with soybeans under treatment (4) represented by CAR2-Mz (Table 1 in the supplementary materials).*"

Comment: *Line 296-337. Add the information and equations for TN.*

Response: Thank you very much for pointing this out. We had modified the section "2.4 Soil organic C analyses", and replaced it by the following version in the main text:

2.4 Soil organic C and total N analyses

The concentrations of SOC and TN of the soil samples collected in 2009 and 2011 were determined by dry combustion using an elemental CN analyzer (TruSpec CN, LECO, St. Joseph, USA). The details of the analysis were described in Hok et al., (2015). Sub-samples of the composite soils (n = 3 per layer) collected in 2021 were finely ground (< 150 µm) before analysis for total C and N by dry combustion using the LECO® CHN628 analyzer at the Sustainable Agroecosystems Lab, ETH Zurich University, Switzerland.

In addition, we modified equation no. (2) in the main text to include the TN stock calculation and replaced it with the following equation:

SOC or TN $stock$ =∑(i=1)^n $[(M_{(soilmin,i)} \times conc._{(i)}) + ((M_{(soil,i)} - M_{(soilmin,i)}) \times conc._{(i-1)})] \times 0.001$ (eq. 2)

Comment: *Line 389-396, 409-413, 428-431, 444-447, 475-478, 497-498. It is recommended to move the results of the RV period to the supplementary materials.*

Response: Thank you very much for your suggestion. Consolidating the first reviewer' comment and your suggestion, instead of moving the results of the "reference vegetation (RV)" period to the supplementary materials, we completely removed all the information, results, and discussion related to "RV" from the manuscript.

Comment: *Line 414-417, 448-452, 499-502. These paragraphs are very unclear, it is recommended to integrate above text with Line 402 - 408, Line 441 - 443 and Line 490 - 496 respectively.*

Response: Thank you very much for your insightful suggestion. We merged the paragraphs in L 414-417, L 448-452, and L 499-502 to above text in the manuscript with Line 402-408, Line 441-443, and Line 490-496 respectively.

Comment: *Line707-719. These sentences and Figure 6 should be moved to the materials and methods to compare the difference between these two approaches or moved to the supplementary materials.*

Response: Thank you very much for pointing this out. As stated in earlier response, we preferred to keep the result of comparison between diachronic and synchronic approaches in the discussion rather than moving it to the supplementary materials.

3. Reviewer's specific comments on Table and Figure

Comment: *Please add standard error for each table and figure.*

Response: Thank you very much for your suggestion. We have added the "values of standard error (+/- SE)" to the Table 3, 4 and 5. For the figures, initially, we also included the error bars (SE) into those graphs, but this makes the graphs become tedious because we cannot distinguish the error bars between the treatments, so we decided to remove them from the graphs. All the raw data are freely available in the dataverse mentioned in the manuscript, so we think colleagues interested into standard errors could directly retrieve them for this dataset.

Comment: ***Table 1.*** *The 'M' should be as 'Mz' in the NT1-Mz, please check and revise.*

Response: Thank you very much for your correction. We apologies for the typo error. We had fixed it.

Comment: ***Table 3, 4 and 5****. It is confusing to understand the difference between uppercase letters (diachronic) and lowercase letters (synchronic) in these tables. It is recommended to keep the results of only one main method (i.e., diachronic), and to include the results of another measurement method (synchronic) in the supplementary materials.*

Response: Thank you very much for pointing this out. We eliminated the results of the synchronic measurement from Tables 3, 4, and 5 and relocated them to the supplemental materials, leaving just the results of the primary measurement, i.e., the diachronic method, on those tables.

Comment: ***Figures.*** *Please add the note in the figure caption to indicate the details of (A), (B), (C), …(E), (F), respectively.*

Response: Thank you very much. We had added a note to the captions of all the figures to indicate the details of each subgraph: (A): Maize-based experiment in 2011, (B): Soybean-based experiment in 2011, (C): Cassava-based in experiment in 2011, (D): Maize-based experiment in 2021, (E): Soybean-based experiment in 2021, and (F): Cassava-based in experiment in 2021.

Comment: ***Fig. 3****. Please add the note to indicate the details of numbers (i.e., 41.6 and 4.32) in this figure.*

Response: Thank you very much for recommendation. In addition to this comment, and integrating the comment from the first reviewer, we had removed the data of RV (reference vegetation) from Figure 3 keeping only the data of PE (pre-

experiment) in 2009 and the data of the four tested treatments in 2011 and 2021. Please note that we run the statistical analysis again with the dataset of PE in 2009, and tested treatments in 2011 and 2021. We also modified the caption of Figure 3, replacing it with the following: “Figure 3. Changes in SOC and N stocks (Mg ha^{-1}) at 0−20 cm depth from pre-experiment (PE) in 2009, 2011, and 2021 under different cropping systems. CTM: monocropping under conventional tillage; CA: conservation agriculture; CAM, CAR1, and CAR2 refer to different cropping CA systems as described in Table 1. Lowercase letters inside the brackets indicate a significant difference between PE and the treatment(s) in 2011 and uppercase letters inside the brackets indicate a significant difference between PE and the treatment(s) in 2021 (Tukey’s test; $P < 0.05$).” Furthermore, we removed all the information, results and discussion related to “RV” from the manuscript.