Response to Reviewer #2

We thank the reviewer for the constructive discussion. We took all the comments very seriously and improved the manuscript further as suggested. In the following the comments of the reviewers are numbered and in cursive followed by our response in plain. Changes in the manuscript can be followed in the revision mode. A new R script was compiled and uploaded to the server. Also S2, the R markdown file was finalized.

The manuscript explores the changes in soil structure and carbon content through different cover crops, it is important for further increasing soil carbon content and improving soil structure. However, this manuscript needs be further revised and improved as shown the following issues:

1. *Although different CCs were selected in this manuscript, what is the basis for selecting these CCs?*

We selected cover crops that reflect several criterion. (1) the CC should be used widely in the practical application. Mustard is one of the cheapest and most widely used CC by farmers in Europe. The same is true for phacelia that is not related to any plant family of cash crops. (2) the plants should represent contrasting plant families with different properties and functional traits. Mustard: *Brassikaceae* with high production of allelopathic substances (like Gucosinolates) also sometimes used to biofumigation approaches. Deep, tab roots until ~1m, high N uptake. Phacelia: *Boraginaceae*, is widely used before sugar beets to reduce nematode pressure. Contained high capacity for P mobilisation from soil sources and high P concentrations in biomass. Bristl oat: *Poaceae*, brings a high rooting density and volume in the upper 50 cm and high N uptake. Egyptian clover: *Fabaceae*, N uptake from atmosphere, low C/N ratio in roots and shoots. Shallow rooting system but high input of rhizosphere products. Mix4 was a mixture of all species at a ratio of 25% each by seeds. Mix12 should represent a maximum of biodiversity with all plant families as above plus some more. The mixture should contain plants with different functional traits to explore niches in soil and nutrients. Further, all CC can be frost killed and do not require herbicide application in regions with cold winters.

2. The role of different CCs was involved in the discussion, but this article does not provide the data of CC. Please supplement these data (such as root morphology, CC carbon type, root density, etc.) to support the discussion;

Plant data, root morphology and root exudation patter are presented in the following publications that were cited in the manuscript:

Gentsch, N., Boy, J., Batalla, J. D. K., Heuermann, D., von Wirén, N., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Céspedes, F. C., and Guggenberger, G.: Catch crop diversity increases rhizosphere carbon input and soil microbial biomass, Biol Fertil Soils, <u>https://doi.org/10.1007/s00374-020-01475-</u><u>8</u>, 2020.

Gentsch, N., Heuermann, D., Boy, J., Schierding, S., von Wirén, N., Schweneker, D., Feuerstein, U., Kümmerer, R., Bauer, B., and Guggenberger, G.: Soil nitrogen and water management by winter-killed catch crops, SOIL, 8, 269–281, <u>https://doi.org/10.5194/soil-8-269-2022</u>, 2022.

Heuermann, D., Gentsch, N., Boy, J., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Guggenberger, G., and Wirén, N. von: Interspecific competition among catch crops modifies vertical root biomass distribution and nitrate scavenging in soils, Sci Rep, 9, 1–11, <u>https://doi.org/10.1038/s41598-019-48060-0</u>, 2019.

Heuermann, D., Gentsch, N., Guggenberger, G., Reinhold-Hurek, B., Schweneker, D., Feuerstein, U., Heuermann, M. C., Groß, J., Kümmerer, R., Bauer, B., and von Wirén, N.: Catch crop mixtures have higher potential for nutrient carry-over than pure stands under changing environments, European Journal of Agronomy, 136, 126504, <u>https://doi.org/10.1016/j.eja.2022.126504</u>, 2022.

Heuermann, D., Döll, S., Schweneker, D., Feuerstein, U., Gentsch, N., and von Wirén, N.: Distinct metabolite classes in root exudates are indicative for field- or hydroponically-grown cover crops, Frontiers in Plant Science, 14, 2023.

All publication produced data from the same set of CC at the same long-term experiment. Therefore, we like to refer to these publications instead of presenting to much details here. We added a paragraph in the material and method section. We also added Table S3 in the supplement on shoot-root biomass and C:N ratios.

3. Please provide field management in the material method;

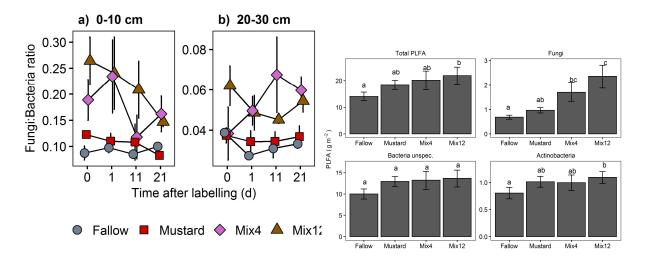
Management is explained in detail in the supplement material S1. But we add a sentence more.

4. Added P values after the significant results, and delete the non-significant results to further refine the results

We added the following sentence: "The cut of between the terms "significant" and "not significant" was defined to p > 0.05." Further p-values were added where needed in the results section and non significant p-values were deleted.

5. The manuscript mentions the contribution of fungi to soil structure, such as L73, L281, etc. However, the number of fungi decreases and the number of bacteria increases due to fertilization in farmland. Moreover, there is no relevant fungal data in this manuscript. Please further revise and improve;

That is right. Farmland has very low Fungi:Bacteria ratios compared to grassland or native vegetation. But it depends on the management. Reduction of soil cultivation increases the Fungal activity and biomass (Helgason et al., 2010), the same for CC applications (Cloutier et al., 2020; Thapa et al., 2021; Gentsch et al., 2020). With respect to fungi we refer in the introduction and discussion to our study (Gentsch et al., 2020) from the same site (see figures below). We found that CC stimulate particularly the fungal activity in the experiments. Fungi:Bacteria ratios increase and also the Fungal Biomass increased during CC growth. The data below based on PLFA measurements during CC growth in October. We found with a ¹³C labelling technique, that fresh photosynthesis C compounds from CC were transported mainly to fungi. We add some more references in the discussion.



6. The figures in the manuscript provide the replicates data, such as Figure 2, and the figures in the supporting information. It is enough to the mean with standard error in the figures. Please further modify and add statistical analysis in the figures;

Unfortunately, we disagree with this suggestion. Just to show means and standard errors is not enough to understand the complexity of the data and the selection of the statistical methods. As explained in the answer of reviewer #1, it is possible so explore the variance and the distribution of the original data and justify the decision to use e.g. Welch's pairwise t-Test. We like to refer to the publication of (Ho et al., 2019), who clearly explained the advantages of data transparency and moving beyond p-values.

7. L252-255 is the result, please move to the results parts; Further refine the discussion section;

The results were already described in the results part in detail. Here we need to call back the reader's attention to the results that we discuss below. We like to refer to the famous book of Schimel (2012), who stated that repeating the most important results is an integral part story telling in the discussion section. These principles are also stated in the academic phrase bank of Manchaster University (<u>https://www.phrasebank.manchester.ac.uk/discussing-findings/</u>). Restarting the results and cantering the discussion around these results is one of the keys of a successful discussion. Each of our discussion paragraphs is following this rule.

8. L292 nuclear magnetic resonance data, not included in the manuscript, please delete it;

Off course we do not show NMR data. We also discuss a lot of microbial related functions and do not show microbial data. Most of the literature we cite used methods that we do not use here. The sense of this section is to discuss data, that relate to our CC study and might explain changes of MWD due to plant inputs. We like to draw the discussion on the importance of OM chemistry for the MWD and aggregation of soil particles. OM chemistry in terms of litter quality is an important factor that that is one of the 4 controls we outlined in the introduction (L54 following). We like to consider all the 4 aspects in the discussion. Without discussion of carbon quality parameters, the discussion would be incomplete. Unless litter C:N ratios and other element stoichiometry we do not have insights to litter quality parameters. Unfortunately, there are not so many new studies on NMR and CC litter quality. But we added some more references to support the discussion (Jensen et al., 2005; Husain and Dijkstra, 2023; Halder et al., 2021; Oliveira et al., 2016).

9. L294 mentions cellulose hemicellulose, please provide relevant data for CC;

We provide several references.

10. Is "the strongest direct effect" in L311 significant?

Yes, shown in Fig.4, numbers show standardized estimates with p values as asterisks.

11. L320 not clear;

Right, it was just a hypothesis that we could not support with data. So, we delete the sentence.

12. Two largest fractions, which fractions?

The 4-8 and 8-16 mm fraction, mentioned in the sentence before. I guess it is clear that the sentences are connected together.

13. Rhizosphere products and hyphae, providing data support

The last paragraph is just a short synopsis of the discussion. Rhizosphere products and fungal impacts are discussed in L285 to 306 with many references. The statement in L352 refers to the reference Tisdall and Oades (1982).

14. Please change the conclusion to a paragraph

Done.

References:

Cloutier, M., Murrell, E., Barbercheck, M., Kaye, J., Finney, D., García-González, I., and Bruns, M.: Fungal community shifts in soils with varied cover crop treatments and edaphic properties, Scientific Reports, 10, https://doi.org/10.1038/s41598-020-63173-7, 2020.

Gentsch, N., Boy, J., Batalla, J. D. K., Heuermann, D., von Wirén, N., Schweneker, D., Feuerstein, U., Groß, J., Bauer, B., Reinhold-Hurek, B., Hurek, T., Céspedes, F. C., and Guggenberger, G.: Catch crop diversity increases rhizosphere carbon input and soil microbial biomass, Biol Fertil Soils, https://doi.org/10.1007/s00374-020-01475-8, 2020.

Halder, M., Liu, S., Zhang, Z. B., Guo, Z. C., and Peng, X. H.: Effects of residue stoichiometric, biochemical and C functional features on soil aggregation during decomposition of eleven organic residues, CATENA, 202, 105288, https://doi.org/10.1016/j.catena.2021.105288, 2021.

Helgason, B. L., Walley, F. L., and Germida, J. J.: No-till soil management increases microbial biomass and alters community profiles in soil aggregates, Appl. Soil Ecol., 46, 390–397, https://doi.org/10.1016/j.apsoil.2010.10.002, 2010.

Ho, J., Tumkaya, T., Aryal, S., Choi, H., and Claridge-Chang, A.: Moving beyond P values: data analysis with estimation graphics, Nat Methods, 16, 565–566, https://doi.org/10.1038/s41592-019-0470-3, 2019.

Husain, H. and Dijkstra, F. A.: The influence of plant residues on soil aggregation and carbon content: A metaanalysis, Journal of Plant Nutrition and Soil Science, 186, 177–187, https://doi.org/10.1002/jpln.202200297, 2023. Jensen, L. S., Salo, T., Palmason, F., Breland, T. A., Henriksen, T. M., Stenberg, B., Pedersen, A., Lundström, C., and Esala, M.: Influence of biochemical quality on C and N mineralisation from a broad variety of plant materials in soil, Plant Soil, 273, 307–326, https://doi.org/10.1007/s11104-004-8128-y, 2005.

Oliveira, R. A. de, Brunetto, G., Loss, A., Gatiboni, L. C., Kürtz, C., Müller Júnior, V., Lovato, P. E., Oliveira, B. S., Souza, M., and Comin, J. J.: Cover Crops Effects on Soil Chemical Properties and Onion Yield, Rev. Bras. Ciênc. Solo, 40, e0150099, https://doi.org/10.1590/18069657rbcs20150099, 2016.

Schimel, J.: Writing science: how to write papers that get cited and proposals that get funded, OUP USA, 2012.

Thapa, V. R., Ghimire, R., Acosta-Martínez, V., Marsalis, M. A., and Schipanski, M. E.: Cover crop biomass and species composition affect soil microbial community structure and enzyme activities in semiarid cropping systems, Applied Soil Ecology, 157, 103735, https://doi.org/10.1016/j.apsoil.2020.103735, 2021.