We would like to thank referee #2 for his thorough review and fair assessment of the first submission. We will incorporate the suggested changes in the revised draft. In a few cases, we explain in detail why we decided against it. Referee comments are reproduced in italic font and answers are highlighted in bold font.

General Comments

This is an original approach to study drivers of decomposition and microbial respiration in soils, in particular soil microstructure. The work is based on clear objectives and a strong sampling design and overall clearly written. I therefore believe this work is worthwhile publishing. The authors find that soil micropore structure as determined with XRTomography does not explain variation in microbial respiration or growth among different land use types, at least not in well aerated soil (NW European spring conditions). They also conclude that POM (particulate organic matter) mainly causes the differences in carbon mineralization among soil types (see title of the paper). My general concerns about this paper mainly relate to this latter conclusion, and in my opinion those concerns need to be addressed through major revision in order to make sure that the results are correctly interpreted and the limitations of the study are understood.

Those concerns include:

- *The interpretation of results based on correlations as proof of causal relationships (see for example the title).*

  We have softened our statements throughout the text and replaced "caused" by "explained variation" or "correlated".

- *The authors make quite strong conclusions on the role of POM in explaining variation in respiration. However it is clear that the methodology used for POM fractionation did not separate root biomass from POM. So it should be very clear that all data and conclusions on the role of POM , in fact correspond with POM + ROOT biomass. This is an artefact of the method because these roots died as a result of sampling. Important to acknowledge this when interpreting your results and the possible implications of this for the interpretation of the results need to be explained clearly.*

  We agree that the reader should be reminded of this experimental shortcoming. We had addressed this already in the original submission but put more emphasis on it in the revised draft, both in the materials & method section and when discussing the data (see actions taken below).

- *Important aspect for interpretation of the results is that "in none of the soil cores the initial water saturation was high enough to induce a deficiency of soil aeration". This is a very important observation that requires attention when drawing general conclusions on the importance of structure for respiration (again, see title). The implications of this for* *the generalization of the results need to be discussed. At the moment this is only brought up at the end of the conclusions section.*

As suggested we have added "in well aerated soils" to the title. The direct quote is taken from the discussion section, not the end of the conclusion section. So we believe that this is exactly, where it should be.

- *The authors should discuss the implications of the fact that the XRTomography has its limitations in terms of resolution (minimum pore size) and also discuss in a bit more depth the potential drivers that could explain mineralization but that were not measured (OM quality, nutrient availability, total SOM, microbial community composition, efficiency). Total SOC data seem to be available but this parameter not included in the regressions as a bulk soil property? Why not?*

The effect of image resolution limit has already been discussed in the previous version. In addition to microbial community structure and carbon use efficiency, which were already discussed as potential drivers, we will now also include OM quality and nutrient availability as unaccounted drivers. C:N ratio and TOC data, were only available through the annual monitoring program as plot averages, but not measured for individual soil cores. This is why we could not include them in the partial least square regression.

Other comments
Title:
The word "caused" is too strong here I believe, was a causal relation really tested? The word "explained" would reflect better the relationships obtained. Also this statement cannot be generalized beyond well-aerated soils. I suggest to change to: "Variation in carbon mineralization across land use types in well aerated soils is mainly explained by particulate organic matter content rather than soil microstructure".

Title will be changed according to your suggestion

Abstract:
Ln 15-16: Please be more specific: This paper is about respiration and microbial activity. Not about the other functions.

For this introductory statement we wish to keep the more general remark about the unclear relationship between soil structure and soil functions, as it follows from the previous statements.

The organization of the abstract can be improved from Ln 22 onwards. First, present the main results that were obtained, and then interpretation, followed by clear conclusions. At the moment results, assumptions and interpretations are all mixed and it is not clear what was actually measured and what was assumed to explain these results. Also be careful with the word "cause" where causal relationships were not established.

We have made an attempt now to restructure this part of the abstract. First we start with the microstructure, POM and respiration results as function of land use. Then we continue with the interpretation of the findings, i.e. the drivers for the variation in respiration. "Caused" has been replaced by "coincided" or "explained":

Ln 24: The word "absent" is too strong here. The effect of moisture cannot be excluded but in the current study these effects may be dominated by other factors such as total SOC? whether this effect is absent should be assessed by manipulating the moisture content of the same treatment and measure respiration. This was (unfortunately) not part of the current methodology.

The word "absent" has been replaced by "not evident". Please see our explanation below, why we did not manipulate soil moisture for basal respiration.

Ln 25-26: How was this concluded from the data presented? No measurements were done in microbial hotspots. This is an assumption not a finding.

Yes, this is an assumption and now clearly stated as such.

Ln 33: I don't think we can exclude that there would be an effect of land use through micropores although the smaller the structure the less likely it is affected by land use.

Yes, we generally agree. But the sentence refers to image-derived microstructural parameters and the meso- and micropores that you are referring to, cannot be resolved.

Ln 38-39: Again, mineralization in microbial hotspots was not measured; this explanation is assumed. Make sure this is understood as the abstract should be understandable as a stand alone text. Also make clear that POM is not only POM, but POM + root biomass.

We have now clearly stated that this is an interpretation and that POM includes root biomass.

Introduction:
Ln 43- 51: This description does not provide a complete picture of the most recent theories on C storage and mineralization - biochemical and physical protection through interactions between organic molecules and reactive soil mineral surfaces is missing.

We have now added a general sentence on this right at the beginning of the introduction: "**Organic C is protected against mineralization by reduced bioavailability through sorption on reactive minerals and physical protection in the soil pore network (Dungait et al., 2012; Schmidt et al., 2011)". When it comes to land use-mediated changes in carbon mineralization further down in the introduction, changes in physical protection are likely to be more relevant than (de)sorption of C on reactive minerals.**

Ln 70 and other places in the manuscript: The authors tend to mix up R and R2. Normally the small letter r is used for correlation coefficient. R2 for goodness of fit of regressions. See also Ln 262-263

In the introduction we have to reproduce what other studies have chosen to report, but will replace R by r according to your suggestion. We agree, that we should use the correct terminology for our own findings. We use R^2 throughout the paper and refer to it now either as explained variability or goodness of fit.

Ln 80-82. The objectives are very clear. But it is not clear to me why you chose to measure basal respiration at field moisture content. To address the objectives wouldn't it have made more sense to test respiration at different moisture contents for the same structure? This would have permitted you to separate microstructural effects from environmental conditions (like soil moisture content).

We see your point but had to decide what was technically feasible. Unifying the soil moisture among all soils cores for basal respiration would have only been possible by raising the water content like it was described for the glucose solution, i.e. full saturation followed by drainage on a sand bed. However, then we could not have done the subsequent growth experiment on glucose anymore with the same set of cores, as they would have been too wet, i.e. capillarity would have been too low to suck up the solution. The basal respiration data, as it is now, might be less controlled, but more a realistic representation of natural conditions, as plots with more vigorous plant growth are necessarily drier. Of course, cutting off roots from plants in order to take intact soil cores to the lab, could be argued to be also quite unrealistic, as we have conceded above. Anyhow, the partial least square regression should have been able to detect at least some predictive power of water saturation in explaining the residual variation of basal respiration that was not explained by POM or any other explanatory variable.

Ln 85: Can you explain what kind of bulk properties you had in mind for this?

Yes, now added (water saturation, bulk density, POM content).

Materials and methods:

Ln 95: No need to mention the part of the experiment with future climate conditions. This is not relevant for the current study.

We agree that it is not relevant for the current study, but i) it belongs to the description of GCEF experimental platform and ii) we refer to it later as an outlook and therefore wish to keep it.

Ln 115: Rather that "initial water saturation" isn't "water filled pore space" a term readers may be more familiar with? I suggest to replace throughout the text and figures.

We agree that water saturation and water-filled pore space can be used interchangeably. Readers with a background in soil hydrology might be more familiar with water saturation, whereas readers with a background soil ecology might prefer WFPS. We think that what matters most is the clear definition of θ/ϕ, i.e. the ratio between water content and porosity, that we keep throughout the paper.

Ln 121-125: The method used to measure POM weight needs better explanation. POM data are quite crucial for the main conclusions of the paper. Make sure it is understood how POM fractionation was done, how the soil was dispersed and if dispersion was complete. Also make sure it is understood that what is measured here as the POM fraction includes root biomass present at the time of sampling. Do you have any idea what was the contribution of root biomass to POM in the different land uses? Did you not measure POM-C? This could provide a more accurate estimate of the amount of POM+rootbiomass?

We agree that in fact a large share of what we extracted via wet sieving in grasslands was fresh root biomass. In croplands it was mostly plant residues from the last seasons. With a rather large mesh size of 0.63 mm we hardly recovered other POM like very fragmented, decomposed residues or biochar.
We did not determine the C content of POM, but since the samples were oven-dried, and the stoichiometric ratio of C mass over total biomass is rather stable, we do not expect an added value. Nevertheless, we will keep this as a good suggestion for future sampling campaigns, as it does not amount to a lot of work. *The complete method description now reads as follows:*

"Finally, soil cores were dispersed during a wet sieving procedure (0.63 mm mesh size) to extract inorganic (sand, stones) and organic (roots, plant litter) components. Inorganic and organic components were subsequently separated by hand and POM mass (m_r) was determined after drying for 48 hours at 70°C. This POM mass does not only include organic material from previous years, but also the fresh root biomass that was cut off during sampling and only started to decay during incubation."

Ln 146: Did the visual method also include root biomass in the POM fraction?

Yes. With X-ray CT and image analysis we were able to resolve POM with an order of magnitude gain in resolution. So in addition to the root biomass and other large organic residues, we were able to resolve smaller POM objects. However, this volumetric information is afflicted with unknown internal porosities in each POM voxel.

Ln 191-200: The PCA analysis is not mentioned in the statistical methods. Also please explain how you dealt with the psuedo-replicates in your models (2 and 3 cores per plot were analysed)?

The biplots are an outcome the partial least square regression (PLSR). The PLSR and principal component analysis (PCA) are both dimension reduction techniques. "PCA […] is applied without the consideration of the correlation between the dependent variable and the independent variables, while PLS is applied based on the correlation." [1]

For PLSR, we chose to treat pseudo-replicates as real replicates. The rationale is now given in the method section:

[1] https://www.casact.org/sites/default/files/database/dpp_dpp08_08dpp76.pdf

"Pseudo-replicates, i.e. the 2 – 3 soil cores from the same plot, were considered individually to explore the full range of variation in target variables. Explanatory variables, which were only available as plot averages (total carbon, total nitrogen) were therefore not considered."

Ln 201-203: Where are all these symbols clearly explained?

Yes, they were all explained at different locations in the method section. In addition, we refer now to Table 1 in which they are listed again with full name and units.

Results
Presentation of results is confusing; please make it easier for the readers to follow. I suggest: Present the results according to the research objectives in a coherent way. What is not directly related to the objective put that in supplement and vice versa. Too many symbols are used in text and graphs. Try to limit them or explain them more clearly.

We appreciate the suggestion, but like to stress that the results have already been structured according to the research objectives, i.e. 1. Comparison of land uses with respect to microstructure and C mineralization. 2. predictive power of microstructural properties and bulk properties on C mineralization. But we will outsource non-essential information like the PLSR biplots into the supplement, also in line with the suggestion of reviewer #1. We agree that the excessive use of symbols is detrimental to readability. However, in the majority of cases we use both the term and the symbol in the text. We have now identified one more sentence, in which we now also use the full term in addition to the acronym. In figures, each symbol is explained in the caption. Adding the term to the axis labels would have often exceeded the space.

Ln 230: Is the volumetric air content after glucose saturation a bulk soil property? What does it tell us? other than the conditions during which SIR was measured?

Yes, it is a bulk property and, yes, it is only relevant for describing the conditions encountered during SIR incubation. It could have been relevant as a proxy for limited oxygen supply, as air diffusivity is known to scale non-linearly with air content. Limited oxygen availability could have had ramifications into microbial activity and metabolic pathways. This is why we reported it. This implication of oxygen supply on microbial growth is now mentioned.

Ln 251: Did both POM measurement methods that were compared include the root biomass?

Yes, both included it.

Ln 252-Ln 255: This belongs to the discussion or to the materials and methods

We prefer to only pick up only those interpretations and discussion in the discussions section that are relevant for the research objectives. This is why we wish to keep these minor

methodological discussions in the result section. A discussion in the material & methods section is suboptimal, as we would also need to present the data then.

Fig 4: Please explain the codes. Graphs should be self-explanatory.
We forgot the put (p_B) after basal respiration in the figure caption. This is added now.

Ln 295-301: This fits better the discussion or conclusions section
Agreed. This paragraph will be moved to the discussion section in the revised paper.

Fig. 6 + 7: PCA model: It is not clear why the PCA was not used to inform the variables to be used in the multiple regression. Why not pick the strongest correlating parameters from PC1, and the strongest correlating parameter from PC2 as test model?

The PLSR biplots have been removed from the main paper. Please note that the explanatory variables with strongest correlations to the target variable are directly identified by PLSR and are considered in the VIP model

Fig. 6a: can the variation in respiration in cropland soils be explained at all? The model with only POM has a R2 of +/- 0.1, so can you say that POM is such a good predictor for respiration across land uses? or only in grassland?
True, the predictive power of POM mass on basal respiration is really bad in croplands. It does a fairly good job in explaining the variability among grassland soil cores and also in explaining the differences in basal respiration between grasslands and croplands in general (R^2 of the pooled dataset).

Fig 6 and 7 do not match with in-text references (fig 7 and 8). It would be nice to have the rational of the different model (e.g. all parameters minus microbial parameters) also explained in captions. Now hard to understand the different models and the ideas of the different models

The numbering of and cross-references to Figs 6 and 7 have been corrected. The rationale is now also explained in the figure caption.

Discussion
The discussion is well organized and generally clearly written, with some exceptions:
Ln 371: "ambient conditions". Please be more specific. Rather than ambient conditions it is better to call this field moisture level. For example temperature was controlled, not ambient.

Changed as suggested.

Ln 376-377: Fertilization/nutrient availability could also be an important explanatory factor, or the C/N of POM.

Picked up as suggested. The soil C:N ratio at the plot level did not differ between land uses. However, nutrient concentrations and C:N ratios of POM may vary at the soil core level.

Ln 386: "..showed that soil compaction reduced soil respiration (Liebig et al., 1995)" -> At what scale? And what moisture levels? In the current study rings were only 100 cm3 and quite dry.

The soil containers in Liebig et al. had a volume of 40cm³ and respiration was measured at three water saturations (47%, 61%, 73% WFPS). Soil respiration was reduced by soil compaction irrespective of water saturation. This important detail will be added to the MS.

Ln 391-395: Good observation about POM including root biomass. But this is a methodological artefact. What are the implications in terms of interpretation? Please discuss.

If soil respiration was measured on-site without cutting the roots, then basal respiration would not have comprised the mineralization of the cut-off roots. The implication would be that our findings would need to be backed up the field measurements to assess the effect. This is now picked up at the end of the discussion section as an outlook.

"Finally, a sizeable amount of basal respiration was likely contributed by the decay of cut-off roots that would not have occurred, if soil respiration was measured on-site. Such field CO_2 measurements with comparable spatial footprint and environmental conditions like in laboratory incubations would be an important step to gauge the effect of intact rhizosphere vs. decaying detritusphere on carbon mineralization."

Ln 401-404: This is not a surprising finding, when considering structural effects. However you did not look at interactions with reactive surfaces so be careful you cannot make any conclusions on this not being a driver of carbon mineralization.

We are not quite sure, if we understand correctly. Would structural effects not speak in favor of abiotic regulation, e.g., through diffusion, and therefore contradict the statement? We agree that the effect of interaction with reactive minerals, i.e. (de)sorption, cannot be studied with our experimental setup. We suggest to soften the statement to account for your comment:
"Our findings indicate that carbon mineralization in well-aerated topsoils that contain fresh POM is biologically driven and mainly governed by carbon availability (Kuzyakov et al., 2009) and less by abiotic processes as proposed by the regulatory gate hypothesis (Kemmitt et al., 2008), at least not by access or diffusion limitation imposed by soil structure."

Ln 405: parameters overlooked could include nutrient availability, POM composition.
Will be picked up in the revised version as follows:

"Local variations in organic matter quality and nutrient availability could also have played a role in carbon mineralization that was not accounted for in the PLSR analysis as that information was not available at the soil core level. For instance, the biodegradability of old crop residues and fresh, cut-off roots are likely to be very different."

Conclusions
Ln 438: Be careful to suggest causal relationships where they were not tested.
Replaced by "explained variation best".

Ln 446-450: Move this part to the Discussion. This is not a good way to end your conclusions section. Finalize with your main conclusion about your objectives, not bringing up a new issue for discussion/reflection.
Agreed. Will be moved to the Discussion section.

Please also note the supplement to this comment:
https://soil.copernicus.org/preprints/soil-2021-56/soil-2021-56-RC3-supplement.pdf

We have addressed all editorial remarks in the commented PDF. In addition we provide brief responses to selected comments:

Line 27: yes, causal logic and a direct consequence of the observation.

Line 410: Unfortunately, we cannot determine CUE properly as we did not recover all CO_2, as the capacity of the NaOH solution to take up CO_2 was exceeded. However, we plan to do this in future sampling campaigns.