Responses to comments from Anonymous Referee #2

In this review, Fromin highlights the necessity and potential pitfalls of various soil storage methods for understanding different microbial parameters. This analysis is an important piece in improving our ability to gain inference from soils that cannot be analyzed immediately after collection. Unfortunately, the analysis here feels quite incomplete for two major reasons:

Thank you for underlining the importance of the subject addressed in this manuscript. I hear and understand your criticisms about the production of this synthesis, and I'm going to try to respond to them.
I propose to prepare a new version of the manuscript in which I will try to take into account the comments below.

#R2.1- The "yes or no" style aggregation of the studies considered in this analysis is not particularly helpful in finding the degree to which soil storage influences microbial parameters. It seems relatively straight forward to intuit that some storage will likely alter the microbiomes in soils, but the important question is "How much?". We must store soils, but understanding if these storage conditions fundamentally change the inference we can gain from them is the critical piece of using these methods. Soil storage may decrease diversity (or some other metric) globally, but if it does this similarly for treatment A and treatment B, that is still a valid method for experimentation. While the author discusses some attributes of effect size in the discussion, greater effort must be taken to quantitatively incorporate (preferably with statistics) effect sizes from these studies into the overall results of this meta-analytical approach.

I completely agree that the size effect is a crucial issue while assessing the significance of storage impacts. I agree that a quantitative study based on effect sizes rather than on the presence / absence / inconsistency of storage effects would have been more informative. My question is: How to quantify these effect sizes in a comparable way across a wide range of metrics (and sometimes at different time steps for the same metric?).
In older articles, the authors frequently show data (microbial parameters) without/with storage as figures, or do not provide the raw data (*e.g.* for all replicates) (the sharing of raw data was not required for the publication of articles). Frequently, authors only report the significance of the storage impacts using ANOVA-based analyses, only mentioning "higher" or "lower values", which makes it impossible to calculate the size effect.
I felt it was more important to see the consistency of the storage effects (microbial parameter that increases/decrease or no impact in all soils, or inconsistent ("variable") effect between the different soils tested) rather than to quantify the storage effects (especially as, as mentioned above, it wasn't always possible). With this approach, I obtained a single data (storage effect) per microbial variable and per storage modality for each study, avoiding to fragment information for easier synthesis.
As you must have realized, it was not my intention to carry out a meta-analysis (this term does not appear in the manuscript), but rather to provide a 'state of the art' of the knowledge about storage impacts on soil microbial properties and to demonstrate the small number of studies and fragmented data available on this subject. It's not interesting to know whether soil storage has an impact on the microbial parameters measured (it usually has an impact), but rather which storage method has the least impact on the analysis of a given microbial parameter, and perhaps dispel some preconceived ideas about best storage practices.

I felt that the information was too fragmented and studies were too dissimilar and then inadequate for a meta-analysis (The average number of studies for a given question, *i.e.* effect of a given storage practice on a given microbial parameter, is low: median = 3.0, mean = 3.78).
As mentioned above, the corpus of studies frequently failed to provide adequate data for inclusion in a meta-analysis (effect size or raw data needed to calculate it, standard errors or confidence intervals), especially in less recent papers. In these conditions, vote counting is the simplest method for synthetizing multiple independent studies.

Finally, because the methods used to characterize microbial parameters have evolved considerably over time, it may be problematic to compare the results of older and more recent studies (*e.g.* for sequence-based variables: sampling effort and sequencing depth are not comparable).
In the forthcoming revised version, I will try to clarify the objectives of the study in the introduction.

#R2.2- The author states several times that the number of studies is too low, however a significant number of papers focusing on this exact issue have been published since 2021. Some of these are referenced in the text but not included in the synthesis. The literature search should be updated to achieve a greater number of studies with which to do analysis and to provide a more compressive, up-to-date view of this field's state.
Indeed, the analysis includes the literature up to 2021. This bibliographical synthesis required a huge amount of time and effort. I recognize that several relevant studies (*i.e.* explicitly comparing microbial parameters in stored *vs* unstored soil samples) have been published since 2021 (most of these studies were not available when I performed the analysis) and must be included in the corpus.
Querying the WOS database again, using the same keywords as those used for the study, gave 204 articles, including six relevant to the synthesis (indicated with an asterisk) (two of these papers, Finn et al. (2023) and Lane et al. (2022), are already mentioned in the manuscript):

* Lane et al. (2022) Soil sample storage conditions impact extracellular enzyme activity and bacterial amplicon diversity metrics in a semi-arid ecosystem. Soil Biology and Biochemistry 175, 108858, https://doi.org/10.1016/j.soilbio.2022.108858
Already mentioned in the manuscript (as additional reference): it will be included in the corpus of the synthesis in the next version.

* Finn et al. (2023) Importance of sample pre-treatments for the DNA-based characterization of microbiomes in cropland and forest soils. Soil Biology and Biochemistry, 184, 109077, https://doi.org/10.1016/j.soilbio.2023.109077
Already mentioned in the manuscript as additional reference. Although the authors present their results as mean relative change in microbial parameters for pre-treated (dried or fresh) soil in comparison to deep-frozen soil (their reference).

*Kushwaha et al. (2024) Field to greenhouse: How stable is the soil microbiome after removal from the field? Microorganisms, 12, 110, https://doi.org/10.3390/microorganisms12010110.

* Lee et al. (2021) Revisiting soil bacterial counting methods: Optimal soil storage and pretreatment methods and comparison of culture-dependent and -independent methods. PLoS ONE, 16, e0246142. https://doi.org/10.1371/journal.pone.0246142.
The authors tested the impact of storage condition on epifluorescence bacterial counts in soil samples stored at -20, 4, or 24 °C in one soil type (pre-study to determine the best storage temperature). This reference will also be cited as an example of pilot study.

* Moy & Nkongolo (2023) Variation in microbial biomass and enzymatic activities in metal contaminated soils during storage at low temperature (4°C). Chemistry and Ecology, 39, 688-709. https://doi.org/10.1080/02757540.2023.2253222
The authors showed that microbial PLFA-based biomass decreased during the first two weeks of COLD storage and remained unchanged thereafter, and that most enzymes inconsistently increased or decreased over time during storage at 4°C.

* Smenderovac et al. (2024) Drying as an effective method to store soil samples for DNA-based microbial community analyses: a comparative study. Scientific Reports, 14, 1725, https://doi.org/10.1038/s41598-023-50541-2
The authors tested several methods for DNA preservation strategy, including freezing at -20°C. Additionally, the authors tested some commercial preservatives, showing that storage at room temperature with silica gel packs gave results compatible to frozen samples. They acknowledge that the preservation methods should be studied on a grater range of soil samples and with more barecodes.

The following 6 references are not retained (I explain why):

Reardon et al. (2022) Enzyme activities distinguish long-term fertilizer effects under different soil storage methods. Applied Soil Ecology, 177, 104518, https://doi.org/10.1016/j.apsoil.2022.104518
Not included because the authors used drying at +40 °C (which is unusual).

Hu et al. (2023) The preservation of bacterial community legacies in archived agricultural soils. Soil & Tillage Research, 231, 105739, https://doi.org/10.1016/j.still.2023.105739
Compared soil samples stored at -80°C or air-dried samples (no comparison with fresh, unstored soils).

Brock et al (2024) Impacts of sample handling and storage conditions on archiving physiologically active soil microbial communities. FEMS Microbiology Letters, 371, fnae044. https://doi.org/10.1093/femsle/fnae044
The 'control' modality was soil samples immediately snap frozen in liquid nitrogen and stored at –80°C. The authors did not compare to non-stored, unfrozen soil.

Pavlovska M., Prekrasna I., Parnikoza I., Dykyi E. (2021) Soil sample preservation strategy affects the microbial community structure. Microbes and Environments, 36, ME20134, https://doi.org/10.1264/jsme2.ME20134
Their control was immediately frozen in liquid nitrogen (no fresh control)

Ouyang et al. (2021) Direct cell extraction from fresh and stored soil samples: Impact on microbial viability and community compositions. Soil Biology and Biochemistry, 155, 108178, https://doi.org/10.1016/j.soilbio.2021.108178
The paper focuses on complex cell extraction procedures before analysis of microbial parameters: this study cannot be compared to other DNA-based studies. The authors showed that cell viability changed and microbial community composition changed in all stored samples, but that the least changes were observed at +4°C. This result can be added in the discussion but not included in the corpus.

Edwards et al. (2024) Long- and short-term soil storage methods other than freezing can be useful for DNA-based microbial community analysis. Soil Biology and Biochemistry, 191, 109329, https://doi.org/10.1016/j.soilbio.2024.109329
The authors compared soils stored under different conditions (e.g; refrigeration versus storage) but not with the same soils immediately analyzed before storage.

Minor comments:

50: *can
This will be corrected in the revised version.

64: this sentence is a bit difficult to read, could it be broken up?
The sentence will be reworded in the next version.

Why are some references using names and some numbers?
As mentioned LL 478-479, the references indicated as numbers are those of the corpus used for the quantitative analysis. The references given by name provide additional information, but the studies do not provide data comparing microbial parameters in stored/non-stored soils.

80: Could help to mention here that DNA is relatively recalcitrant, thus potentially less susceptible to storage effects than other microbial parameters
Based on the literature review, I wouldn't say that DNA is "relatively recalcitrant", but that it is "deemed less recalcitrant than other biomolecules such as PLFAs or exoenzymes". This will be mentioned in the revised version.

Table 1: This information might also be more useful as a figure, maybe a bar graph with date as the X axis and number of studies as the Y, exp as you have the same info in Table 2.

The data presented in Tables 1 and 2 are not the same. Table 1 shows the evolution over time of the methodological approaches used to characterize the impact of storage practices (with an increasing proportion of DNA-based studies), while Table 2 summarizes this impact over the whole period (1961-2021) and by storage practice. Thank you for your suggestion for your suggestion to present the data of Table 1 in graph form (a suggestion shared by Referee 2). This will be done in the revised version.

Table 2: I believe this info would also be more digestible in some graphical context

I agree that Tables 1 and 2 are relatively indigestible. The choice of presenting data by listing study references (which is not possible in a graph) enables readers to immediately identify and refer to studies of interest (impact of a storage practice on a microbial parameter). I'm thinking about how to present this data in graphical form (at the moment, I can't see how to do it).

361: *are preserved

This will be corrected in the revised version.

366: Difficult sentence, too many clauses

I propose to rephrase the sentence as follows:

"*As anticipated, the impacts of storage practices differed between microbial parameters, with frequent inconsistent effects reported across different studies. As a result, it seems tricky to advocate for one best storage practice for a given microbial parameter*."

446: Storage cannot be avoided. It is impossible to measure these characteristics in situ, thus, this is not a realistic or helpful recommendation for from this analysis.

I would say that "storage of soil samples cannot *always* be avoided" (particularly in the case of distant study sites or large numbers of samples). My conclusion was "*These results suggest that storage should be avoided* ***whenever possible****, and especially when different soil types or soils of various geographical origins are compared*." In the conclusion, I also follow the conclusion of Rhymes and coll. (2020) who recommends limiting the duration of storage as much as possible, and conducing a systematic pilot study to assess the impact of storage on the studied soils (LL454-457). The output of such pilot studies should be included in the publications (L458).

One additional conclusion would be that authors working on pre-stored soil samples should systematically mention possible storage-related biases. The objective of this synthesis is to provide them with contextualized elements.