nature portfolio

Peer Review File

Microbial trait multifunctionality drives soil organic matter formation potential

Corresponding Author: Dr Emily Whalen

This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
Review NCOMMS-23-61270

General comments
Whalen et al. present an interesting study on the effects of different fungal isolates on soil organic matter (SOM) formation. They measured a wide range of fungal traits in 8 isolates, and correlated individual traits, as well as a measure of multifunctionality with the potential of these fungi to form SOM in artificial soil systems. The analyses of fungal traits and soil C properties are impressively comprehensive, with carefully considered methodological details. The writing and presentation of the study is well done, it was a pleasure to read. The topic is very timely, regarding the current relevance of microbial traits in soil ecology, as well as the rapidly developing field of soil organic carbon sequestration.
I have several minor comments listed below. Unfortunately, though, there is one big concern which needs to be addressed by the authors. Fungi were incubated in soil with a growth medium containing glucose and potato infusion extract. This is generally no problem (though it will be interesting in future studies to analyse the effects of more complex C sources on the patterns observed). However, while reading and trying to understand the results, I started to wonder what happened to unused glucose/medium-derived C in the analyses of SOM. A defined amount of medium C was added to each soil, and incubated with fungal isolates, which were quite different in growth rates, for a defined period of time (adjusted to fungal growth dynamics). In order to analyse C residues at the end of incubation, soil was simply air-dried (L600), and total C determined, as well as C in MAOM and POM fractions. Additionally, different C fractions (varying in degree of chemical stability) of these samples were determined. In a subsequent step, the biological stability of SOM was determined, incubating soils again with a microbial community.
All of these methods are excellent. Still, it is unlikely that fungal isolates used all of the glucose (and potato infusion) provided in the incubation. Especially fungi with low growth rates likely used less of the C provided, which would explain that the trait growth rate negatively correlated with total C contents: faster growing fungi use more glucose, i.e., partially transform it into CO2 leading to soil C losses (and less total soil C). Similarly, the analysis of biologically stable SOM will be strongly influenced by leftover glucose in soil, since glucose would stimulate microbial growth much stronger than more recalcitrant fungal necromass/residues.
I would be interested in the opinion of the authors on this topic. And whether this methodological bias could somehow be eliminated in the analyses. I also thought about a better methodological design, and have to admit that there is none. The only alternative would be to inoculate soils with fungal residuals/necromass without growth medium, but that would lead to a more artificial system, since residues formed during fungal growth would not be added to SOM.
Also, the detailed chemical analyses of SOM, and the chemical-stable fraction would not be biased by glucose. It may be an option to primarily focus on those results.

Specific comments
L100 soil microbial CUE (or isolate-specific CUE)?
L120-143 This last paragraph is written in unusual style. Instead of formulating clear hypotheses, the authors provide a summary of the most important results. I suggest to check with the journal guidelines again.
L429 What is the evidence for this statement. Is it based on sequencing results, or mushroom data?
L461/462 is “quart-size” a general volume which is known to readers (it is not to me, so maybe adding the exact volume would be helpful)?
L465/466 I understand the rationale of the authors to decide for different measuring times, depending on isolate-specific

growth. However, this decision has pros and cons. One could also argue that growth time would strongly affect the C sequestration dynamics, and the time constant is an important factor in real soils, where there is no differentiation in time-dependent processes among species.
I think based on the question – testing the effects of fungal traits on SOM - this decision can be justified. But it is important to do right at the beginning (here). Otherwise, in the next passages lots of questions arise whether this decision was useful, and how it may or may not impact the methods and results.
L474 Please specify “regularly”
L475 please add “the time of substrate amendments”. It can be confused with different amounts of C added to each isolate
L478 Was this the baseling of activity? A figure of respiration curves of individual isolates in the supplements would be very helpful. With an overview of times (not necessarily for each isolate, rather in general) for C application times.
L479 better give days?
L484/485 More of personal interest, what was the start n? So how many samples were contaminated during this time period? We also have problems to do long term incubations with regular gas measurements.
And were the jars kept open inbetween the CO2 measurements to avoid O2 limitations?
L487 Please also give the full name of the genus for this isolate
L501 It may be worth mentioning here or in the discussion that this final hyphal length does not reflect the total hyphal length produced by a fungus, due to fungal recycling mechanisms. It is great that the authors additionally used other metrics for growth rates.
L516 Same medium as used for the main experiment?
L517 Please specify how growth rate in liquid culture was defined. Biomass produced after (which?) period of time?
L518 Please add in brackets how the growth phase was monitored, here or in L520. Based on respiration data?
L532 Is 4.5 the conversion factor? Then a unit is missing?
L533-535 This is very difficult to understand. Neither the formula for the definition of growth rate is clear (very relevant to understand the results), nor the description of the growth curves produced. Maybe present some of these linear curves in the Supplement to clarify?
L567 Soils of which experiment?
L612 What was the original aggregation status of the artificial soils. No WSA?
L646 Please add the rationale of adding 13C glucose. To differentiate from 12C fungal derives SOM? Can this be clearly separated in this design? Some 12C may be added by the inoculum.
And why 25 atom % instead of 99?
L648 Please spell out FDIW
L658 “control” in this case refers to soils previously incubated without fungi?
L710 Please spell out MSE
L716 Since multifunctionality is one of the key results in this study, I would like to understand its exact meaning. Intuitively I would think it is the number of functions fulfilled, as well as the strength of the function. So the more and the stronger, the higher the multifunctionality,. Basically the bigger the circle in Fig. 1? However, the term “uniformity” suggests that the metric is calculated differently? (Also not clear to me from going quickly through the cited reference). Please clarify.
L181 Is it clear how much of the glucose was consumed? These numbers seem to refer to C (SOM) remaining in soil after respiratory losses. I just wonder how much of that C is fungus vs. glucose, is it possible to distinguish between these C pools?
L207-210 This finding supports the notion that fast growing isolates formed more biomass/necromass, which was biologically stable, while slow growing isolates likely used less of the medium C provided.

Fig. 1: I like this plot! Only point is that the colour variation for the different fungal species is confusing since also the different traits have a colour variation. The meaning of colours should be clarified. In this plot I would personally prefer grey tones, however, I understand the species can be more easily compared with the other figures when keeping the colour differentiation.
Also in this case it would be possible to add full species names on top of the graphs, so readers can directly see differences among isolates.
The colour gradient is also not strong enough to differentiate different species in Fig. 4, 5 and 6.
But looking at the supplements I do understand now that the colour gradient reflects multifunctionality, is that correct? In this case it is a useful gradient, but this should be clearly mentioned in the legend.
Fig. S5, S6, S10 are of low quality. please check.

Reviewer #2

(Remarks to the Author)
The present manuscript aimed at describing microbial traits that predict soil organic matter (SOM) formation. The authors used model soils and 8 fungal strains to quantify in the lab several physiological parameters such as Carbon Use efficiency (CUE), fungal growth, enzyme production, along with the formation and stability of soil particles. In addition, the authors assess how the degree of microbial trait multifunctionality affects SOM formation and stability. This is an interesting, relevant, lab-intensive, and ambitious approach. One of the most interesting finding is that fungal species with the highest SOM formation potential are also responsible for generating more chemically diverse and stable SOM.
The strength of this manuscript is that it provides and relates, using adequate analysis, multiple measurements that support the abovementioned findings.
However, the manuscript needs to be developed, the introduction is vague, repetitive and requires structure and clearer and simpler words. There are many subjective terms in quotation marks that need to be replaced or at least defined. The results

text is missing most of the statistic results, particularly results between species (Tukey HSD) which I find essential, and the text is vague. I would like the respiration data to be included.
The discussion could benefit of more detail, and more context with other relevant work in the field.

Specific comments:

Introduction
L65 – Functional pools are defined in the following sentence so I think quotation marks should be removed.
L65-85 – these paragraphs include very long sentences; I suggest rewriting the text and using smaller sentences. In addition, the two paragraphs include some repetition just with slightly different words as for example L65-66 and L79-80.
L80 – what is meant by “functional complexity” and why is it written with quotation marks? Is it the same as “functional pools” in line 65? This needs clarification.
L75 – for clarity, the text between brackets should be written without the use of symbols.
L77-78 and L82 – these two sentences are repetitive in the sense that they start with “emerging understanding” and “emerging view”, please rephrase for improvement. In addition, I would like the authors to comment whether these are so novel and emerging views. The cited references are from 2020 and 2021 and have been cited at least 334 and 5392 times. I consider that these views are widely mentioned and considered in relevant literature, and that there is plenty of ongoing research on these topics. For those reasons I would not consider it an “emerging view”, instead I would rather argue that these certainly need further development and empirical support. I would like to know what the authors think about this specific point.
L92-93 – This is a confusing definition of CUE. CUE is partitioning of organic carbon used for growth to organic C taken up and used for both growth and respiration. Please check definitions by other authors and re-write the sentence for clarity.
L97-98 – This sentence is vague and unclear. What do the author mean with “singularly dominant traits or binary trade-offs between traits”? Please rephrase for clarity.
L 105 –The referred study is one example of what the authors say is “emerging evidence”. Are there are other studies backing up the emerging evidence mentioned here, also in other biomes/ experimental systems?
L109 – In this context there are better words in the English language than “feedstock”, which according to the Oxford Dictionary, is a raw material used to power a machine or an industrial process. If a synonym that applies to the context here is used instead the authors can avoid the use of quotation marks. Please revise this throughout the text for clarity and simplicity.
L110 – what are “formation traits” and why is it written in quotation marks? Can it be defined, or another choice of words made? Please revise throughout the text.
L111 – it is unclear what is meant with “approximate”, please revise for clarity.
L120 – please replace “microbial with “fungal”.
L114 and 123 – I would like the authors to explain what is meant by “holistic approach” particularly with regards to their study and methodology (the use of a model soil and 8 fungal isolates). What is the holistic approach of this study and how does that holistically contribute to an increased understanding of SOM formation? This should be added to the main text.
L126 – I would like the authors to explain the choice of reference number 39 here. The cited study investigates soil C accumulation under nitrogen enrichment in one temperate forest, and where C accumulation seems to result from the suppression of decomposition… In this study, fungi dominate the microbial biomass pool, but is it saprotrophic or mycorrhizal fungi? How is this reference supporting the broad statement that fungi dominate the microbial biomass pool? How does that influence your choice of methods? Can you mention other references and add to the text?
L129 – I would like the authors to argue for the possibilities and representativity of studying SOM formation potential in a soil-free-model soil.
L132 – what challenges are these?
L137 – “higher fungal growth rates”, higher in comparison to what? How were the respiration rates in those cases? Higher than the compared term? If not, then CUE should be higher (than..) too.
L140 – what is meant with “multifunctional” and why the use of quotation marks? Are there single-functional species? If yes, please give examples with references. The authors write that “These multifunctional species also formed SOM that was more chemically diverse”, in comparison to what? This needs clarification.
L143 – what hypotheses were tested?

Results
L153 – here the authors mention MANOVA but in the Methods section where statistics are described there is no mention to MANOVA. This needs to be clarified.
L155 – what are intermediate values? This is unclear. It would also be helpful to refer to the traits that are related to degree of specialization.
L153-158 - extremely vague section, please add values, and more details about what traits are referred to. For instance, the authors write that P.stipticus and Gymnopus sp. exhibit a greater degree of specialization, greater in comparison to what? The authors add that “high values restricted to a more limited suite of traits”. What traits are these? Please be specific. I would like the results section to follow this suggestion, that the authors refer to which traits specifically, which values, and what they are comparing.
L159 - here the authors mention MANOVA but in the Methods section where statistics are described there is no mention to MANOVA. This needs to be clarified. How were the differences between species tested?
L160 – The authors write “may be phylogenetically conserved”. This is 1) an interpretation and therefore should not be in the results section; 2) not directly connected to any measurement performed in the study and therefore 3) highly speculative. Please remove from the text.
L169-170 – what statistics were used here? There is one p value for the whole paragraph and multiple comparisons are mentioned. How were differences between species tested? Please report P values for every statement/comparison.

L171 – Please write in the text what enzymes specifically you refer to so it can be checked in the Figures. Was this tested? What statistics were used and what p values were obtained?
L186 – Can the authors present the results of the statistical test?
L188 – was this significant, please present the statistical test.
192 – total SOM or Total C?
L195 – PHOS seem to have a higher VIP value than phenol. Perhaps it should be included in the tex?
L201 – There is no mention to WSA. Please add information on this.
L208 – the relationships described here are not linear, what function did the authors fit, was it a quadratic function? Can the authors claim that PC1 is associated with greater chemically and biologically stable C in a parabola? Is this due to differences between Ascomycota and Mucoromycotina? Please be precise and specific and add enough detail and be cautious in your interpretations.
L212 – what do the authors mean with intermediate performance, please describe specifically, and refer to figures and statistical tests. From PCA and PLSR it is difficult to draw such conclusions. And I also question what this subjective term means.
L217-218 – is the hypothesis in L211-214 accepted or rejected?
L224 – Refer to the figure where this data is presented.
L226 – what is an intermediate CUE? How were these categories defined and in comparison to what is this intermediate?
L230 – what figure here?
L234 – what is fungal identity? Do the authors mean whether there are differences between species in terms of their effect on SOM chemistry? I suggest using clearer and more precise language. Please rephrase for clarity.
L255 – does this mean that if there is a higher number of chemical compounds the proportion of chemically stable C is higher? How is this explained versus the perhaps strong contribution of a few compounds?
L260 – Did your estimates of CUE make sense? How do they compare to other studies? For instance, CUE of 0.8 is often considered the theoretical maximum. Please provide some context to your findings.
L265 – how did respiration look like? Please present the respiration data.
L265-266 – so is it species with high multifunctionality or with intermediate performance? This is rather confusing. And what species were these?
L273 – aggregate formation is not mentioned in the results section, please add.
L290 – what were the traits that contributed the most to SOM formation? Have similar results been found by other studies? Please provide context.
L300 – CUE was, in the PLSR model, one of the most important traits contributing to SOM formation. How is CUE related to being a generalist and having intermediate trait multifunctionality? Can we learn something about physiological and genetic trade-offs? I suggest the following read:
Saifuddin, M., et al. (2019). "Microbial carbon use efficiency predicted from genome-scale metabolic models." Nature Communications 10.
L303 – What is meant by “bundles of functions with synergies”? Please use clearer terms and avoid the use of subjective expressions in quotation marks.
L307 – can the authors clarify what is a “single community level syndrome”?
L321 – can you mention a couple of reasons why intermediate SOM formation would amplify SOM formation potential?
L317-326 - How did respiration rates looked like? Can that explain some of these findings?
L337 – what is meant by activity levels? Is it growth? Respiration? Please be specific.
L344 – how would this be different from high CUE with high growth and low respiration?
L346 – what is meant by “field soils”? environmental soil samples versus model soils?
L351 – what is the size of this fraction, was it quantified?
L354 – does more active fungal species mean intermediate? The choice of words is vague. More active in comparison to what?
L392 – This reference is also context dependant. Could the authors provide what the differences are in other contexts with references?
L 394 – Did the authors find any no-multifunctional microbes? If not, then this sentence needs to be re-written.
L 402 – But wasn’t CUE the most important contributor to SOM formation? So why intermediate?
L 403-406 – does the data support this statement? Please provide details with mentions to figures.
L412 – I think that the use of a model soil and the measurements in this manuscript should be carefully described as accounting for pathways by which microbial residues are incorporated into SOM pools. I would like the authors to comment on this.

Methods
In the Guide for authors it is clearly stated that “for all statistics (including error bars), provide the EXACT n values used to calculate the statistics (reporting individual values rather than a range if n varied among experiments).” I don’t think the authors have reported this. Please check and add the information.
L458 – was this a one-time addition?
L459 – is this soil CN ratio similar to that of Harvard forest?
L472 – is the choice of 840 mg C based on a published study, or how was this calculated?
L 473 – where is the Respiration data? I cannot find it in the main manuscript or supplementary material. This data is important because it balances growth in CUE. The authors refer in the results section to higher or lower growth but do not mention respiration. Please provide the data and include it in your results and discussion where useful.
L478 – what is this choice of respiration rate based on?
L485 – how many replicates were included exactly?
L523 – how was respiration measured here?
L595 – what is “(5)”? and what is the conversion factor used? Please include that in the text.

L536 – Can you explain in detail how is respiration obtained by the slope?
L537 – I would like the author to explain the use of standard error in a model soil with 8 fungal isolates since the standard error measures how far the sample mean (average) of the data is likely to be from the true population mean. What would your true population be and how representative are your experimental systems to a true population?
L 565 – is this mentioned in the discussion, or are these results presented and discussed without mention?
L686 – what R package was used for PLSR?
L 698 – add that VIP stands for Variable Importance in Projection, this is present in the Figure 4 caption but should be in the main text too. Also, VIPs are often used as variable selection, and this involves setting a VIP value threshold. What value did the authors choose for variable selection?

Reviewer #3

(Remarks to the Author)
The manuscript of Whalen et al. presents an interesting approach to derive insights on the interactions of microbial traits with soil organic matter transformation. The authors used a simple model system with sand grains and montmorillonite particles incubated axenically with various fungal species isolated from a hardwood forest. Glucose and potato infusion extract were used to provide C and ammonium nitrate to provide N. The substrate was added three times at 5.6 mg C g-1 amounting to 16.8 mg C g-1 over the course of 3-6 months. The authors evaluated various fungal traits and properties of the remaining model soil to evaluate the fungal-derived organic residues. A multifunctionality indicator is computed and correlated with the residue properties.
The manuscript provides novel detailed insights that promote the understanding of soil organic matter dynamics. However, various major aspects should be considered:
• The performance of individual fungi isolated form a hardwood forest to utilize glucose does not represent the transformation of more complex plant litter, since the fungal species originate from an ecosystem where specialized traits to decompose litter derived from the hardwood vegetation and the ability to cooperate are more pronounced. The limitations of using glucose as a model substrate are not adequately addressed (e.g. l. 348). Could the fungi that turned out to perform as multifunctionalists in this manuscript (l. 295) simply be more specialized in glucose consumption? It is questionable to which extent insights on species-specific organic matter transformation (l. 348) can be inferred since the adaptation to glucose is reported to suppress the enzymatic activity for the assimilation of other C sources.
• The data seems to indicate that the recycling of the fungi's own biomass, as seen for example by the increased phosphatase enzymatic activity, plays an important role (l. 230, l. 341). It is not clear whether this observation is affected by the variation of incubation times ranging from 3 to 6 months. The necromass/biomass recycling affects the evaluation of the carbon use efficiency, which describes the partitioning of carbon for biomass growth versus respiration. Two different approaches were used in the manuscript to evaluate the CUE and it is not clear which one the text is referring to when mentioned in the results (l. 192). This aspect is especially important to clarify since the manuscript claims to demonstrate that "CUE alone is inadequate to predict the formation of SOM" (l. 403).
• It should be noted that bacteria were not included at all, i.e. "understanding the role of microbial traits" (l. 267) is limited to fungal traits only.
• The production of different fungal-derived protein and phenol contents is quite interesting. It might be advisable to strengthen the conclusions on this part, e.g. extend the finding that "the source of many soil phenols is unknown" (l. 372). However, here it is advisable to consider older literature, which was strongly focused on phenols form plant or microbial, and especially fungal origin as precursors in SOM formation.
• The context dependency (l. 392) and the modulation of trait activity by soil properties (l. 389) are not clear.
• There does not seem to be a clear pattern related to MOM vs. POM formation of fungal residues. (Fig. 3). It is necessary to report the recovery of C and N in the fractions of their bulk contents (l. 603).
• It is necessary to come up with clear messages derived from the incubation experiments, rather than quite non-descript claims for a more "holistic approach". This is especially necessary, as "pathways by which microbial residues are incorporated into different SOM pools" have been studied among others with isotopic approaches are extensively reported in the literature.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

The authors significantly revised the manuscript, addressing all Reviewer comments thoroughly. I have few comments on the revised Manuscript, and one more comment addressing one of the answers. I am sure the authors can integrate these suggestions, and I strongly encourage Publication of the study in Nature Communications.
I thank the authors for their thoughtful answer to my comments on glucose leftovers influencing the results. Looking at Fig. S3, I agree that it seems likely that glucose was used and respired completely by all fungal isolates in this experimental design. I still find it an important point, since it cannot be completely ruled out that there are leftovers of medium components, and this point must be carefully considered in future designs. In fact, I have already seen and been involved in experiments planned with a similar design, and depending on conditions and research questions glucose may be problematic, as well as other medium components like proteins. In case there is another limiting factor to growth, e.g., an environmental stressor that may limit fungal growth and/or another element limitation in certain isolates by N, P or even micronutrients. For this reason, I find it important to mention such potential biases in the main text, not only in the Supplements. This point should be clearly discussed either in the Methods or Discussion sections.

Supplementary Material:
L99: It may also be an option to determine the amount of C lossed via CO2? There are likely lower and upper limits to CUE of fungi, so the C lossed should be relatively comparable among isolates in case all isolates used glucose. Though I agree, there will be different types of recycling of material and high variation in efficiencies. But it may be a good estimate whether values are comparable over time.
L151-153 To put this text into context, it would be helpful to add the formulas and response variables used for different CUE estimates.
Main Manuscript:
Fig. 4B It may be helpful to indicate in the x-axis the differences in the parameters. This way it looks like three plots are showing exactly the same
Fig. 4A Maybe I missed it, what is the unit of chem- and bio-stable C; is it a proportion?

Reviewer #2

(Remarks to the Author)
General comments:
The present manuscript aimed at describing microbial traits that predict soil organic matter (SOM) formation. The authors used model soils and 8 fungal strains to quantify in the lab several physiological parameters such as Carbon Use efficiency (CUE), fungal growth, enzyme production, along with the formation and stability of soil particles. In addition, the authors assess how the degree of microbial trait multifunctionality affects SOM formation and stability. This is an interesting, relevant, lab-intensive, and ambitious approach. One of the most interesting finding is that fungal species with the highest SOM formation potential are also responsible for generating more chemically diverse and stable SOM. The strength of this manuscript is that it provides and relates, using adequate analysis, multiple measurements that support the abovementioned findings.
However, after reading the manuscript again I believe it has some issues, not in terms of methodology but in the ways it moves the field forward. The study lacks context in field soils, and the authors do not succeed in comparing their findings to the multitude of papers on SOM formation that are published. In addition, the significance of the study to the field is limited by the impossibility to generalize their results without a field trial. This study needs to be complemented with a field study exploring how trait multifunctionality can explain soil formation and stabilization in natural environments.
The manuscript still includes too much jargon and terms in brackets that do not add anything to the text. The discussion is superficial, lacks structure and clarity. The introduction is vague and the authors present one hypothesis which is a very unspecific research question. The unnecessary jargon and terms in brackets need to be removed (see specific comments), and clearer text will improve the readability.
Also, abbreviations throughout the text need to be checked. Please add units in the axis of each figure, same for tables, and not in captions, it makes interpretation more difficult. Figures need improvement as most results are presented by species and the results presented by phylum, and the color coding used does not work.

Specific comments
Line 64 - replace soil organic matter with SOM, already introduced in l49. Check throughout the manuscript
Line 93-98 – The commonly accepted explanation for the coupling between high CUE and SOM formation is because higher CUE entails higher allocation of resources to growth and biomass formation, and that microbial necromass, due to its chemical composition, remains in soil and thus contributes to SOM formation. The authors fail to explain this important link that I consider essential, and I suggest rephrasing because this is briefly mentioned in lines 128-129 but should be introduced earlier on.
Line 108 – I have commented on this before and I am not convinced with the response given by the authors. The word “feedstock” is, as the authors replied, a terminology specifically introduced by Sokol et al. (2022) which has not been widely adopted. Sokol and his co-authors (which are authoring also this paper I am reviewing) will understand the term, and perhaps few others, but as mentioned, “feedstock” has not been widely adopted. The reason why I disagree with its use is because in an introduction the ideas/concepts are more important for the reader than the use of specific words. The authors are adding confusion and unnecessary jargon, and I would like to stress again that clarity of ideas is more important than the use of vague and unfamiliar terms. Same for “formation traits” in line 123.

Line 129– there is no reference to why different fungal species were used, I think this should be included because the results are organized in that way, and this is stressed by the authors in lines155-156. Statistical results are presented for differences between phyla, so there must be a reason for this. It is unclear if there are expectations towards how different species contribute to SOM formation differently, and whether there are specific hypothesis connected to different species/phyla? Please clarify.
Line135-138 – the hypothesis is vague and the expected relationship between the variables and outcome is unclear. Please re-write in a concise and simple way, a hypothesis is a prediction and should be objective and testable.
Line 166– I think this needs to be toned down, I agree that Basidiomycota and Mucoromycotina are grouped and separated, but Ascomycota are quite spread along PC1 in Figure 2.
Line 166-184 – Throughout the manuscript it is difficult to follow Figures where measurements for each fungal species are presented but the text in the results section and some tables refer to fungal species grouped in Phyla. The color coding does not help as there is no explanation in the figure or caption. Please improve the figures and instead of color coding (or along) add what Phylum each species belong to.
Lines 167-168- by comparing different Phyla the authors do an ANOVA with groups with unequal sample sizes. Could this have affected the robustness of ANOVA and therefore your conclusions?
Line 231 – is GR introduced before? Please check.
Line 248-249 –In these lines the authors refer to the 5 fungal species with highest SOM formation potential stating that they are associated with higher SOM formation. It seems that this relationship doesn't hold, because in Fig. 1 Gymnopus sp have high trait values for CUE and turnover but low SOM formation potential in Fig. S2B. I would like to hear the authors comment on this.
Line 257 – the word "different" is missing before "fungal species"
Line 287-288 – It is widely accepted and that different microbial traits contribute to SOM formation and complexity. This paper adds empirical evidence that it is so, but it is an overstatement to say that the paper is "proposing that synergies among these traits promote SOM quantity, stability and functional complexity". Please remove this.
Line 307-309 – First this sentence sounds circular to me, the authors present two explanations for the differences between fungal species in terms of SOM formation: the first is the variation in fungal traits (l308); the second is that differences in SOM formation were linked to variation in fungal traits. Please rephrase and remove repetition. Second, this is a very general sentence for a discussion. In which way did the variation in pathways for fungal-derived SOM affect SOM formation? I suggest being more specific about how fungal species differently contributed to SOM formation based on your own results.
L210-311 – why is this not hypothesized previously? Perhaps the choice of the different fungal species had this in mind? As I commented before, it is necessary to state why the choice of different species, grouping according to Phyla in the results section, and whether the authors had the generalists vs specialist framework already in mind.
L313 – would this also apply to species with low investment in trait categories such as Panellus stipticus, is this a generalist too? Which of the species are specialists and generalists according to the framework used by the authors, and how was this assigned? What is the rationale behind picking intermediate level multifunctionality as a generalist trait and not low ? This seems quite arbitrary from the text, it is important that the author clarify this.
L317 – 318 – this is speculation, the data does not test for whether differences in multifunctionality are linked to genetic traits, and importantly, the authors do not build up this argument with other studies. So this statement needs support from relevant literature.
L319-320 – I might have missed it, so could the authors use their own data to support that different fungal traits were associated with the formation of each SOM functional pool? Again, this is extremely general and does not seem to point towards any specific result in the study.
L322 – The authors write that "bundles of functions with synergies" expresses a novel ecological concept by Fiedler et al. (2021). But in the paper by Fiedler et al. (2021), this term is used only once throughout the text. The word "bundles" is used two more times as "bundles of services" and "different bundles could be integrated across the landscape". I do not understand what is the new ecological concept "bundles of functions with synergies" the authors refer to when Fiedler at al. (2002) do not refer to it as an ecological concept themselves. "Bundles" means a collection of things, and that is how the words as been used 3 times in that paper. So, this is not a new ecological concept in the paper by Fiedler at all (2021) and this term is introduction confusion and lack of clarity in the discussion. If the authors want to write "bundles of functions with synergies" I suggest the authors with their own words. Please remove this expression in brackets to improve readability in the manuscript.
L326 – Please explain what is meant by a "community level syndrome". A syndrome is a collection of symptoms, a combination of things. I don't understand what is meant here or the reply by the authors. In the paper by Malik et al 2019 (reference 28) the word "syndrome" is never used. This is, again, unnecessary jargon that decreases clarity in the manuscript.
L327 – 330 – the authors here criticize the use of binary views such as "high vs low" overemphasizes extreme ends. What do the authors propose instead in this paper? A view with three levels, "low", "intermediate" and "high". How, and using own data can the authors argue that this is a better framework?
L337 – 340 – Please provide an explanation for this sentence. Why is SOM amplified at intermediate CUE?
L337-348 – So is it intermediate (l339-340) or high (l347) trait multifunctionality that lead to higher SOM formation? It seems like it is both but in the end of this set of sentences the author seem to conclude that it is the high trait multifunctionality that leads to SOM formation.
L 354 – "where C inputs are relatively continuous", please add one or two examples
L 359 – 362 – so greater SOM formation potential (l359) was associated with higher necromass decomposition (L360) due to increased mineral-associated SOM? How can the authors connect their own data with mineral reactivity? Also, can the authors explain what is meant by diffusion of residues to soil minerals? The sentence needs rephrasing for clarity.
L 365 – why would that not lead to stable C forms? Is it because different microbial residues are formed, or what is the explanation? Could this be just species specific, in other words, related to the biomass chemical composition and not to functional traits? Is it CUE that is the core of the discussion or is it turnover rate or chemical composition instead?

L377-381 – Does this agree with existing literature or is it a unique finding of the study? Please add that to the text.
L424-427 – this paper does not include plant compounds and their contribution to SOM formation, so it is difficult to understand why the authors try to integrate their results with the contribution of plant material. That has not been tested and does not link well to the previous sentences.
L429-431 The field and readers would benefit from more specific recommendations for future research.

Reviewer #3

(Remarks to the Author)
The manuscript has been generally well improved during the revision. Additional justification regarding the usage of glucose as a model compound, further clarified usage of the CUE, an improved terminology, and a strengthened discussion on the microbial production of phenols, among other aspects, have been advanced in the revised version. Since the manuscript aims to conclude on the formation of soil organic matter, it should be highlighted again that the recovery of MAOM-C and POM-C as proportion of their bulk contents are required (l. 640, Fig. 3b and c, Fig. S4e and f) to assess the representability of these functional pools and their discussion based on correlations with microbial properties.

Reviewer #4

(Remarks to the Author)
I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

Version 2:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
I am satisfied with all the changes and responses to my comments. Congratulations for your work!

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Note to Reviewers:

Thank you very much for the opportunity to re-submit our manuscript. We are very grateful for the excellent suggestions made by each reviewer—we feel that the revised version of our manuscript has been strengthened considerably based on their feedback. Below we detail how we addressed each reviewer's overarching suggestions as well as their specific line edits.

Reviewer #1 (Remarks to the Author):

General comments
Whalen et al. present an interesting study on the effects of different fungal isolates on soil organic matter (SOM) formation. They measured a wide range of fungal traits in 8 isolates, and correlated individual traits, as well as a measure of multifunctionality with the potential of these fungi to form SOM in artificial soil systems. The analyses of fungal traits and soil C properties are impressively comprehensive, with carefully considered methodological details. The writing and presentation of the study is well done, it was a pleasure to read. The topic is very timely, regarding the current relevance of microbial traits in soil ecology, as well as the rapidly developing field of soil organic carbon sequestration.

We thank this reviewer for their encouraging feedback that our study is "impressively comprehensive" with "carefully considered methodological details," and that our paper "was a pleasure to read."

I have several minor comments listed below. Unfortunately, though, there is one big concern which needs to be addressed by the authors. Fungi were incubated in soil with a growth medium containing glucose and potato infusion extract. This is generally no problem (though it will be interesting in future studies to analyse the effects of more complex C sources on the patterns observed). However, while reading and trying to understand the results, I started to wonder what happened to unused glucose/medium-derived C in the analyses of SOM. A defined amount of medium C was added to each soil, and incubated with fungal isolates, which were quite different in growth rates, for a defined period of time (adjusted to fungal growth dynamics). In order to analyse C residues at the end of incubation, soil was simply air-dried (L600), and total C determined, as well as C in MAOM and POM fractions. Additionally, different C fractions (varying in degree of chemical stability) of these samples were determined. In a subsequent step, the biological stability of SOM was determined, incubating soils again with a microbial community.
All of these methods are excellent. Still, it is unlikely that fungal isolates used all of the glucose (and potato infusion) provided in the incubation. Especially fungi with low growth rates likely used less of the C provided, which would explain that the trait growth rate negatively correlated with total C contents: faster growing fungi use more glucose, i.e., partially transform it into CO2 leading to soil C losses (and less total soil C). Similarly, the analysis of biologically stable SOM will be strongly influenced by leftover glucose in soil, since glucose would stimulate microbial growth much stronger than more recalcitrant fungal necromass/residues.
I would be interested in the opinion of the authors on this topic. And whether this methodological bias could somehow be eliminated in the analyses. I also thought about a better methodological design, and have to admit that there is none. The only alternative would be to

inoculate soils with fungal residuals/necromass without growth medium, but that would lead to a more artificial system, since residues formed during fungal growth would not be added to SOM. Also, the detailed chemical analyses of SOM, and the chemical-stable fraction would not be biased by glucose. It may be an option to primarily focus on those results.

Thank you for raising this important point, which we did not address clearly enough in our original draft. In the context of laboratory incubations, if more substrate-C is added than can be utilized by a microbial population over the length of the experiment, then there is the potential for unutilized substrate-C to remain in the soil. However, we do not believe this is an issue with our experiment due to careful design and timing of our substrate additions and incubation harvests. First, glucose has been shown to be rapidly assimilated and metabolized by a wide range of microbial taxa (Hill et al., 2008), including saprotrophic fungi (Griffiths et al., 1998; Rousk & Baath, 2011). Compared to other C-supplying substrates, glucose exhibits low levels of discrimination among microbial taxa (Jones et al., 2018), and it has therefore been utilized in numerous incubation experiments as a primary C source.

Second, we focused on developing a study design that would promote maximum substrate utilization by fungal isolates, such that we could assume a majority of the substrate-C had been utilized by each species prior to incubation harvest. Our approach was based on monitoring respiration rates, and either waiting to add subsequent substrate amendments once respiration had declined to < 1 μg CO_2-C g^{-1} soil $hour^{-1}$, or intentionally inducing a period of substrate limitation to encourage biomass recycling (during which times respiration remained low at < 1 μg CO_2-C g^{-1} soil $hour^{-1}$). The length of time that it took each isolate to meet these conditions determined the total incubation length (ranging from 3-6 months across isolates). To avoid potential confusion and provide a stronger rationale for this approach, we have (1) added a new supplementary methods section that provides a more detailed description and rationale for this aspect of our experimental design (see 'Supplementary methods: Key experimental design decisions & rationale'); and (2) we added the respiration data to the supplementary materials (Fig. S3), which provides needed context for this approach (e.g., demonstrates that a respiration rate of < 1 μg CO_2-C g^{-1} soil $hour^{-1}$ is low [near-zero] relative to the peak respiration values observed for fungal isolates following substrate additions). Corresponding line edits that were made to the text are addressed below ("specific comments" section).

Specific comments
L100 soil microbial CUE (or isolate-specific CUE)?

We were referring more broadly to "soil microbial CUE." As suggested, we added this at L100.

L120-143 This last paragraph is written in unusual style. Instead of formulating clear hypotheses, the authors provide a summary of the most important results. I suggest to check with the journal guidelines again.

We had structured this section with a summary of primary results based on examples of prior publications in *Nature Communications*, many of which included a results summary in the last paragraph of the introduction. We reviewed additional examples and re-structured the last

paragraph of our Introduction accordingly. The revised version includes a hypothesis statement, followed by a shortened summary of our key results (L137-144).

L429 What is the evidence for this statement. Is it based on sequencing results, or mushroom data?

This is based on meta-barcoding (sequencing) data. We added text to clarify this (L450).

L461/462 is "quart-size" a general volume which is known to readers (it is not to me, so maybe adding the exact volume would be helpful)?

We added the specific volume (~946 ml) to the text (L486).

L465/466 I understand the rationale of the authors to decide for different measuring times, depending on isolate-specific growth. However, this decision has pros and cons. One could also argue that growth time would strongly affect the C sequestration dynamics, and the time constant is an important factor in real soils, where there is no differentiation in time-dependent processes among species. I think based on the question – testing the effects of fungal traits on SOM - this decision can be justified. But it is important to do right at the beginning (here). Otherwise, in the next passages lots of questions arise whether this decision was useful, and how it may or may not impact the methods and results.

Thank you for this feedback. As suggested, we added a sentence to the end of this paragraph to address the possibility of other standardization approaches (e.g., standardized by time) and to provide a rationale for our selected approach (L506-509 of revised manuscript). We also added a longer rationale/justification for our selected approach to a new supplementary methods section, and we refer readers to that section for additional discussion on this topic.

L474 Please specify "regularly"

Respiration was monitored every 12-96 hours, depending on isolate growth stage. We added this detail to the text at L498.

L475 please add "the time of substrate amendments". It can be confused with different amounts of C added to each isolate

As suggested, we edited this sentence as follows (L499-500): "…the timing of substrate amendments was determined on an isolate-specific basis…"

L478 Was this the baseline of activity? A figure of respiration curves of individual isolates in the supplements would be very helpful. With an overview of times (not necessarily for each isolate, rather in general) for C application times.

As mentioned above, a respiration rate value of 1 μg CO_2-C g^{-1} soil $hour^{-1}$ was very low relative to peak respiration rates measured following substrate additions for each isolate. Therefore, it was very close to the baseline of activity. As suggested, we added a new supplementary figure

(Fig. S3) showing these respiration curves for each isolate, indicating the timing of substrate amendments.

L479 better give days?

As suggested, we changed this to "~20 days" rather than 500 hours (L504).

L484/485 More of personal interest, what was the start n? So how many samples were contaminated during this time period? We also have problems to do long term incubations with regular gas measurements. And were the jars kept open in between the CO2 measurements to avoid O2 limitations?

We added this information to the new supplementary methods section. We established eight replicates per isolate, such that sufficient replication would remain in case of contamination. For some of the faster-growing species, contamination was not a significant challenge and all eight replicates remained at the end of the incubation experiment. For slower growing Basidiomycota that took longer to establish initial growth (e.g., *P. stipticus*), we experienced more significant contamination challenges (only 3-6 replicates remaining). We did not open jars between respiration measurements. We kept jars sealed and flushed the jars with CO_2-free air using needles fitted with 0.2 μm filters to reduce/prevent contamination. Contamination is certainly a challenge with any longer-term culture-based experiments that require O_2 replenishment and repeated substrate amendments. We conducted multiple pilot experiments before establishing the final experiment to determine the methods that best reduced and prevented contamination (described in new supplementary methods section).

L487 Please also give the full name of the genus for this isolate

We added the full genus name (*Hypocrea*) to the text (L516 of revised manuscript).

L501 It may be worth mentioning here or in the discussion that this final hyphal length does not reflect the total hyphal length produced by a fungus, due to fungal recycling mechanisms. It is great that the authors additionally used other metrics for growth rates.

As suggested, we added a sentence at L538-540 recognizing this possibility.

L516 Same medium as used for the main experiment?

Yes, the same media solution was used as the main experiment. We added this detail to L549.

L517 Please specify how growth rate in liquid culture was defined. Biomass produced after (which?) period of time?

As requested, we added this information. It was combined with the revision that we made to address the following comment (see below).

L518 Please add in brackets how the growth phase was monitored, here or in L520. Based on respiration data?

We added the following text to address this comment and the previous comment (L549-552): "Fungal biomass was collected through vacuum filtration and weighed to assess growth, while respiration was monitored using the LI-COR infrared gas analyzer. Mass-specific growth rate was calculated as biomass-C produced biomass-C^{-1} hr^{-1}."

L532 Is 4.5 the conversion factor? Then a unit is missing?

The published conversion factor was 5.0 to convert μg DNA g^{-1} soil to μg MBC g^{-1} soil (Anderson and Martens, 2013). Based on this comment and a similar comment from Reviewer 2, we added this detail to the text at L564. This conversion factor was determined by a regression-based analysis, where 5.0 was the slope of the line representing the relationship between μg DNA g^{-1} soil and μg MBC g^{-1} soil. The average conversion value calculated in our study was 4.5, which was similar to this published conversion factor. We hope that the revision we made at L564 helps to clarify this point.

L533-535 This is very difficult to understand. Neither the formula for the definition of growth rate is clear (very relevant to understand the results), nor the description of the growth curves produced. Maybe present some of these linear curves in the Supplement to clarify?

As requested above, we more clearly defined how growth rate was measured in liquid culture (see edits at L549-552). In addition, we revised the text at L568-570 to further clarify how growth rates were calculated. We regressed the log of microbial biomass carbon values over time and took the slope of the linear portion of these growth curves to estimate growth rates (consistent with standard practice for measuring log-phase growth and as described in Morrison et al., 2022 [ref. #32]). The slope of this line represents the rate of change in MBC over time.

L567 Soils of which experiment?

We added a sentence at L604-606 to clarify which soils were analyzed for potential enzyme activities.

L612 What was the original aggregation status of the artificial soils. No WSA?

Yes, the artificial/model soils were initially non-aggregated (no WSA). We added the following sentence at L650-653 to clarify: "The model soil mixture began as a combination of un-aggregated particle separates (sand, silt, clay), such that any aggregates that formed could be attributed to the growth and activity of the fungal isolates."

L646 Please add the rationale of adding 13C glucose. To differentiate from 12C fungal derives SOM? Can this be clearly separated in this design? Some 12C may be added by the inoculum. And why 25 atom % instead of 99?

The purpose of the biological stability assay was to assess the relative stability of the SOM generated by each isolate against decomposition by a mixed microbial community. Similar to priming experiments, which add ^{13}C-labeled substrates (often glucose) to evaluate microbial decomposition of native SOM, using the ^{13}C-labeled glucose allowed us to differentiate the loss of ^{12}C-CO_2 from soils (which would have been from fungal-derived SOM) vs. the respiration of ^{13}C-CO_2 from the newly added ^{13}C-glucose. We added text at L687-691 to clarify this rationale.

L648 Please spell out FDIW

We spell out "filtered deionized water (FDIW)" at L582, and we use this abbreviation throughout the Methods section (five instances). Therefore, we chose to leave "FDIW" abbreviated in this instance as the term has already been defined.

L658 "control" in this case refers to soils previously incubated without fungi?

Yes, the control samples refer to the samples of artificial soils that received the same amount of substrate media but were not inoculated with fungi. To ensure this is clear, we specified that these are "uninoculated control samples" at L680.

L710 Please spell out MSE

As suggested, we removed this abbreviation and spelled out "mean square error" (L757).

L716 Since multifunctionality is one of the key results in this study, I would like to understand its exact meaning. Intuitively I would think it is the number of functions fulfilled, as well as the strength of the function. So the more and the stronger, the higher the multifunctionality,. Basically the bigger the circle in Fig. 1? However, the term "uniformity" suggests that the metric is calculated differently? (Also not clear to me from going quickly through the cited reference). Please clarify.

We added a broad definition for multifunctionality (following Byrnes et al., 2014 [ref. #45]) at L760-761 and clarified what was meant by "uniformity." This sentence now reads: "…effective multifunctionality, which accounts for both the total provisioning of functions as well as the evenness (e.g., relative trait values) of those provisions of function (Byrnes et al., 2023)." In addition, we added a short definition of multifunctionality at the beginning of the Results section where we first mention this term/calculation (L153-154).

L181 Is it clear how much of the glucose was consumed? These numbers seem to refer to C (SOM) remaining in soil after respiratory losses. I just wonder how much of that C is fungus vs. glucose, is it possible to distinguish between these C pools?

Please see our previous response above to this overarching comment/concern at the beginning of this document.

L207-210 This finding supports the notion that fast growing isolates formed more

biomass/necromass, which was biologically stable, while slow growing isolates likely used less of the medium C provided.

This comment is also related to the reviewer's overarching comment/concern about glucose utilization by fungal isolates, especially whether it is possible that the slowest growing isolates utilized less of the added substrate-C than the fastest growing isolates. We addressed this comment above in our full response, where we detail why we do not believe this is an issue in our experiment, due to the strategic timing of experimental substrate additions and harvests.

While the two slowest-growing isolates (Basidiomycota) did have the lowest values for biologically and chemically stable C, the results were more complex than this. One of these isolates (*Gymnopus sp.)* had the highest MAOM value of any of the isolates, and both of the Basidiomycota species had high water-stable aggregate production relative to some of the faster-growing species. Additionally, *Gymnopus sp.* had the highest hyphal length of any isolate (measured in soils at the end of the long-term incubations), suggestive of high biomass production. Because we standardized the length of incubations to ensure near-complete substrate utilization across isolates (e.g., the two Basidiomycota were incubated for ~6 months, whereas some of the faster growing species were incubated for ~3 months), we believe that these differential contributions to SOM pools reflect differences in the whole (multidimensional) trait profiles of the eight fungal isolates and their net effects on SOM formation.

Fig. 1: I like this plot! Only point is that the colour variation for the different fungal species is confusing since also the different traits have a colour variation. The meaning of colours should be clarified. In this plot I would personally prefer grey tones, however, I understand the species can be more easily compared with the other figures when keeping the colour differentiation. Also in this case it would be possible to add full species names on top of the graphs, so readers can directly see differences among isolates.

As suggested, we removed the color variation for the trait categories to prevent confusion; the trait labels are now presented in all black. Additionally, we added the full species names rather than their abbreviations as labels in Fig. 1. To improve clarity, we also added that "the color associated with each fungal species is carried throughout subsequent plots" to the figure legend.

The colour gradient is also not strong enough to differentiate different species in Fig. 4, 5 and 6. But looking at the supplements I do understand now that the colour gradient reflects multifunctionality, is that correct? In this case it is a useful gradient, but this should be clearly mentioned in the legend.

Thanks for this suggestion. As requested, we added this detail to the legends for Fig. 1 and Fig. 4. In general, trait multifunctionality and SOM formation potential do increase from light to dark colors in these figure legends; however, in order to use the same color palette across figures, we chose to have each color associated with a specific fungal isolate (categorical) rather than representing a continuous value for trait multifunctionality or SOM formation potential. We hope that the revisions/additions of text we made to the figure captions help to clarify this.

Fig. S5, S6, S10 are of low quality. please check.

As requested we replaced these lower quality figures with high quality figures (now Figs. S6, S7, S11). It appears that there was some loss of quality after importing to MS Word in our original submission.

Reviewer #2 (Remarks to the Author):

The present manuscript aimed at describing microbial traits that predict soil organic matter (SOM) formation. The authors used model soils and 8 fungal strains to quantify in the lab several physiological parameters such as Carbon Use efficiency (CUE), fungal growth, enzyme production, along with the formation and stability of soil particles. In addition, the authors assess how the degree of microbial trait multifunctionality affects SOM formation and stability. This is an interesting, relevant, lab-intensive, and ambitious approach. One of the most interesting finding is that fungal species with the highest SOM formation potential are also responsible for generating more chemically diverse and stable SOM.
The strength of this manuscript is that it provides and relates, using adequate analysis, multiple measurements that support the abovementioned findings.

We are glad to hear that this reviewer found our manuscript and experimental approach to be interesting, relevant, and ambitious, and that overall, the reviewer found the analytical components (both laboratory and data analyses) to be robust in supporting our findings.

However, the manuscript needs to be developed, the introduction is vague, repetitive and requires structure and clearer and simpler words. There are many subjective terms in quotation marks that need to be replaced or at least defined. The results text is missing most of the statistic results, particularly results between species (Tukey HSD) which I find essential, and the text is vague. I would like the respiration data to be included.
The discussion could benefit of more detail, and more context with other relevant work in the field.

Thank you for this feedback. We have carefully reviewed this reviewer's specific comments below, and we have addressed these suggestions to the best of our ability. We feel that the clarity and specificity of the manuscript has been improved by addressing these comments.

Specific comments:

Introduction
L65 – Functional pools are defined in the following sentence so I think quotation marks should be removed.

We removed the quotation marks around functional pools (L65).

L65-85 – these paragraphs include very long sentences; I suggest rewriting the text and using smaller sentences. In addition, the two paragraphs include some repetition just with slightly different words as for example L65-66 and L79-80.

As suggested, we shortened some of the sentences in this section (separating longer sentences into two separate sentences, e.g., L66-71). In terms of the reviewer's comment about repetition, L65-66 describes the functional pools that comprise SOM (e.g., POM and MAOM), whereas L79-80 describes Lehmann et al. (2020)'s concept of SOM 'functional complexity.' Because this term refers to a specific theory around the role of SOM spatial heterogeneity and molecular diversity in promoting SOM retention/persistence in soils (described in L79-80), we view this concept as distinct, and we do not believe it is repetitive to the text at L65-66.

L80 – what is meant by "functional complexity" and why is it written with quotation marks? Is it the same as "functional pools" in line 65? This needs clarification.

As defined at L79-80, "functional complexity" is a concept proposed by Lehmann et al. (2020) (reference #17) to describe the spatial heterogeneity and molecular diversity of SOM. They propose that greater spatial heterogeneity and molecular diversity (i.e., greater SOM functional complexity) promote the persistence of SOM in soils. This is distinct from the general term "functional pools," which is used to describe the different pools (e.g., POM and MAOM) that comprise SOM. Please see response above.

L75 – for clarity, the text between brackets should be written without the use of symbols.

For clarity and simplicity, we removed the text in brackets at L75 (previously read: "MAOM > stable microaggregates > macroaggregates > POM"). We felt that spelling out "greater than" in place of the symbols would have made the sentence too long, and removing the symbols altogether would have made the passage unclear. We decided that the text in brackets was not essential to the meaning of the sentence, and thus removed it.

L77-78 and L82 – these two sentences are repetitive in the sense that they start with "emerging understanding" and "emerging view", please rephrase for improvement. In addition, I would like the authors to comment whether these are so novel and emerging views. The cited references are from 2020 and 2021 and have been cited at least 334 and 5392 times. I consider that these views are widely mentioned and considered in relevant literature, and that there is plenty of ongoing research on these topics. For those reasons I would not consider it an "emerging view", instead I would rather argue that these certainly need further development and empirical support. I would like to know what the authors think about this specific point.

To reduce repetition, we removed the second instance of "emerging," such that the sentence now reads "This view underscores the need for…" (L82).

The reviewer also raises the point about whether the two cited papers (Lehmann et al., 2020 and Kleber et al., 2021) indeed represent an emerging view, given they have been widely cited (in our searches, citation numbers appear to be 445 and 358, respectively). Both papers were published in *Nature* journals and have received widespread attention. However, in the case of Lehmann et al. (2020), the functional complexity framework is still discussed as a hypothesis within the field, and empirical tests of this framework are still rare. In the case of the Kleber et al. (2021) paper, our understanding is that a central aim of this paper was to challenge widespread and persistent assumptions within our subfield around the long-term stability of the

MAOM pool, despite a large body of evidence documenting the diversity of OM turnover times within this pool. While there is now greater awareness that the MAOM pool is complex and OM is associated with minerals via multiple binding mechanisms with a range of strengths, Kleber et al. (2021) emphasized the need to reorient our field to viewing these bonds as temporary. Experimental and observational studies of MAOM as a proxy for "stable" or "persistent" SOM are still common within our field, and there are relatively few studies that have quantified more persistent subfractions of the MAOM pool. For these reasons, we believe that the ideas and theories presented in Lehmann et al. (2020) and Kleber et al. (2021) still represent an "emerging view" in need of greater empirical support. This context is important framing for the more comprehensive set of measurements that we made in our experiment to characterize the complexity of SOM produced by fungi.

L92-93 – This is a confusing definition of CUE. CUE is partitioning of organic carbon used for growth to organic C taken up and used for both growth and respiration. Please check definitions by other authors and re-write the sentence for clarity.

As suggested, we reworded this sentence for clarity. It now reads: "…the proportion of C that microbes allocate towards growth relative to respiration" (L92-93). This definition is consistent with common definitions proposed in the literature (e.g., Geyer et al., 2019; Manzoni et al., 2012).

L97-98 – This sentence is vague and unclear. What do the author mean with "singularly dominant traits or binary trade-offs between traits"? Please rephrase for clarity.

We rephrased this sentence for clarity. It now reads: "However, evidence is mixed, suggesting that current trait-based frameworks emphasizing single traits (e.g., CUE) – or binary tradeoffs between traits – are insufficient to describe the microbial controls on SOM accumulation." The mention of "binary tradeoffs" in this sentence refers to the "genetic and/or physiological tradeoffs" discussed at L95.

L 105 –The referred study is one example of what the authors say is "emerging evidence". Are there are other studies backing up the emerging evidence mentioned here, also in other biomes/ experimental systems?

A recent paper, published last month in *Soil Biology and Biochemistry* by Sokol et al. (2024), demonstrates that CUE was negatively associated with new MAOM-C formation in both rhizosphere and detritusphere soils, but this negative effect was strongest in detritusphere soils where a larger proportion of MAOM-C was directly plant-derived. We added a reference to this publication at L105.

L109 – In this context there are better words in the English language than "feedstock", which according to the Oxford Dictionary, is a raw material used to power a machine or an industrial process. If a synonym that applies to the context here is used instead the authors can avoid the use of quotation marks. Please revise this throughout the text for clarity and simplicity.

The use of the terms “feedstock traits” and “formation traits” refer to a specific trait-based framework put forward by Sokol et al. (2022). We use these terms in quotation marks to refer to the specific terminology proposed in this publication, in part because they are terms that have not yet been widely adopted within the subfield. We found these concepts useful to reference in the introductory framing for our study, as they describe different possible categories of fungal traits related to SOM formation—those traits that best predict total microbial residue (“feedstock”) production in soils, and those traits that best predict the incorporation of microbial residues into relatively stable pools of SOM (“MAOM formation traits”; *sensu* Sokol et al., 2022). We have clarified the source of this terminology by including an additional reference to the Sokol et al. (2022) publication after the initial mention of “feedstock” at L109.

L110 – what are “formation traits” and why is it written in quotation marks? Can it be defined, or another choice of words made? Please revise throughout the text.

We hope that our response above has helped to clarify that the terms “feedstock traits” and “formation traits” are based on a recent conceptual framework proposed by Sokol et al. (2022). Formation traits are defined as those microbial traits that influence the subset of microbial residues that become stabilized in soils from the total pool of residues (“feedstock”) that is produced by the microbial community. Examples may include fungal hyphal morphology and necromass residue chemistry.

L111 – it is unclear what is meant with “approximate”, please revise for clarity.

For clarity, we replaced “approximate” with “predict” (L112).

L120 – please replace “microbial with “fungal”.

Later in the manuscript, we replaced an instance of “microbial” with “fungal” when we were referring to the specific results of this study (L288). In the results and discussion sections, we take care to emphasize that our results are based on a study system involving fungal isolates only (no bacteria); however, we do think that our results hold implications for broader understanding of the relationships between SOM formation and microbial traits, as all of the measured traits in this study are also observed among bacteria. In the introduction (including L120), we begin with a broader overview of knowledge gaps in understanding of the role of microbial traits in SOM formation. At L127-128, we describe our study system involving fungal isolates incubated in model soils, introducing the focus on fungi. Therefore, we chose to leave the broader term “microbial” at L121, given that the focus on fungi is introduced shortly thereafter.

L114 and 123 – I would like the authors to explain what is meant by “holistic approach” particularly with regards to their study and methodology (the use of a model soil and 8 fungal isolates). What is the holistic approach of this study and how does that holistically contribute to an increased understanding of SOM formation? This should be added to the main text.

In the original version of the manuscript, we used the term “holistic approach” to refer to an approach that more comprehensively accounts for (1) the formation of multiple SOM pools and (2) multiple microbial traits that may be important for SOM formation. As we discus in the

introduction, most studies that have examined relationships between microbial traits and SOM formation have either focused on individual traits (e.g., CUE) or have focused on the formation of a limited number of SOM pools (e.g., only total SOM, or more recently total SOM and a single measure of MAOM). For clarity, we replaced "holistic approach" with "comprehensive approach" (e.g., L115, L125) and added the following text at L124-126: "We took a comprehensive approach, accounting for the role of these multidimensional trait profiles in the formation of multiple SOM pools, including total SOM-C, POM, MAOM…"

L126 – I would like the authors to explain the choice of reference number 39 here. The cited study investigates soil C accumulation under nitrogen enrichment in one temperate forest, and where C accumulation seems to result from the suppression of decomposition… In this study, fungi dominate the microbial biomass pool, but is it saprotrophic or mycorrhizal fungi? How is this reference supporting the broad statement that fungi dominate the microbial biomass pool? How does that influence your choice of methods? Can you mention other references and add to the text?

Thank you for pointing this out. The second citation listed here (He et al. 2020, Global biogeography of fungal and bacterial carbon in topsoil) is a broader reference providing support for this statement. This paper found that fungi dominate the microbial biomass C pool (relative to bacterial biomass C) in many global ecosystems. We replaced reference #39 with another global-scale analysis (Yu et al., 2022) that analyzed >3000 observations of fungal and bacterial relative abundances in soils, finding that fungal dominance increases in cold and high-latitude environments, and in ecosystems with high soil C contents (e.g., boreal and temperate forests). This line edit can be found at L128 in the revised manuscript.

L129 – I would like the authors to argue for the possibilities and representativity of studying SOM formation potential in a soil-free-model soil.

As suggested, we added a stronger rationale for this approach at L132-137, providing references to past studies that have demonstrated these model systems are relevant to studying microbial SOM formation (e.g., Pronk et al., 2013, 2017; Kallenbach et al., 2016; Del Valle et al., 2022).

L132 – what challenges are these?

Please see previous response above. We revised this section for clarity, and the specific reference to "challenges" has been removed.

L137 – "higher fungal growth rates", higher in comparison to what? How were the respiration rates in those cases? Higher than the compared term? If not, then CUE should be higher (than..) too.

We revised the concluding paragraph of the introduction based on comments from this reviewer and others. The reference to "higher fungal growth rates" has been removed from the revised manuscript. Related to the reviewer's question above, we added respiration data to the supplement (Fig. S3). This addition is addressed in more detail in our responses to other reviewer comments.

L140 – what is meant with “multifunctional” and why the use of quotation marks? Are there single-functional species? If yes, please give examples with references. The authors write that “These multifunctional species also formed SOM that was more chemically diverse”, in comparison to what? This needs clarification.

In the revised version, we removed this reference to “multifunctional” species. The term multifunctional is now introduced for the first time in the Results section, where it is defined more explicitly (e.g., L152).

L143 – what hypotheses were tested?

In the revised manuscript, we added a hypothesis statement at L137-139.

Results

L153 – here the authors mention MANOVA but in the Methods section where statistics are described there is no mention to MANOVA. This needs to be clarified.

Thank you for pointing this out. We added a sentence in the Methods describing the MANOVA analysis (L753-754).

L155 – what are intermediate values? This is unclear. It would also be helpful to refer to the traits that are related to degree of specialization.

We added text at L156-160 describing that the results we present represent the relative trait values of the eight fungal isolates (relative to one another) under the experimental conditions of our study. We hope this helps to clarify what is meant by “intermediate” or “high” trait values in this context (i.e., if an isolate has an “intermediate” value for a given trait, it had a value that fell within the middle range of values exhibited by the eight isolates).

L153-158 - extremely vague section, please add values, and more details about what traits are referred to. For instance, the authors write that P.stipticus and Gymnopus sp. exhibit a greater degree of specialization, greater in comparison to what? The authors add that “high values restricted to a more limited suite of traits”. What traits are these? Please be specific. I would like the results section to follow this suggestion, that the authors refer to which traits specifically, which values, and what they are comparing.

The purpose of this sentence (previously L153-158, now 162-166) is to describe a broad pattern that we observed across isolates; thus, we feel that adding additional detail comparing individual species and their relative trait values would detract from the broader point that we aim to make.

That said, we believe that the revisions we made above in response to this reviewer’s comments have improved the clarity of our comparisons across isolates. For example, when we say that certain taxa exhibited a greater degree of specialization (describing these as taxa with “high

values restricted to a more limited suite of traits"), we are comparing the relative trait values of isolates exhibited across their trait profiles (Fig. 1). To improve the clarity of this section, we made the following additional changes: (1) we added an additional reference to Fig. 1 at L163, which is both a quantitative and visual representation of relative trait values and profiles of each fungal isolate; and (2) we added additional text at L166-167 as follows: "Correspondingly, these species had relatively low values across the remaining suite of measured traits (Fig. 1, Fig. S2)."

L159 - here the authors mention MANOVA but in the Methods section where statistics are described there is no mention to MANOVA. This needs to be clarified. How were the differences between species tested?

Thank you for pointing this out. We added a sentence in the Methods describing the MANOVA analysis (L725-726). This text clarifies that MANOVA was used to test for differences among species.

L160 – The authors write "may be phylogenetically conserved". This is 1) an interpretation and therefore should not be in the results section; 2) not directly connected to any measurement performed in the study and therefore 3) highly speculative. Please remove from the text.

As suggested, we removed this from the text (L168 of revised manuscript).

L169-170 – what statistics were used here? There is one p value for the whole paragraph and multiple comparisons are mentioned. How were differences between species tested? Please report P values for every statement/comparison.

Thank you for this feedback. Due to the high number of fungal traits and SOM pools measured in this study, we aimed to simplify the text of the Results section by referencing Fig. S1 (trait values) and Fig. 3 (SOM pools), which include p-values from one-way ANOVAs comparing trait or SOM pool values across species. Based on this reviewer's comments, we realized that this approach was not comprehensive enough, as these figures do not list the one-way ANOVA results for phylum-level comparisons, nor do they present the results of Tukey HSD tests for pairwise comparisons between individual phyla. As such, we added the respective p-values to the text throughout this section (L169-182) and we state which statistical tests were used (with appropriate figure references).

Additionally, we added two supplementary tables (Table S1 and S2) listing the results/p-values for one-way ANOVA or Kruskal-Wallis (non-parametric) tests at the phylum-level, as well as p-values from individual pairwise comparisons (Tukey HSD or Dunn tests) between individual phyla. Table S1 is for trait values and Table S2 is for SOM pools.

L171 – Please write in the text what enzymes specifically you refer to so it can be checked in the Figures. Was this tested? What statistics were used and what p values were obtained?

As suggested, we added the names (abbreviations) of each enzyme to the text (now L180-186). P-values have also been added for the one-way ANOVA or Kruskal-Wallis analyses comparing

phyla and a new supplementary table has been added summarizing the results of pairwise phylum-level comparisons, based on post-hoc Tukey HSD or Dunn tests (Table S1).

L186 – Can the authors present the results of the statistical test?

We added the p-values for the results of the one-way ANOVAs (or Kruskal-Wallis tests; non-parametric data) for each of the listed SOM pools to the text (starting at L200), and we added a reference to the new supplementary table that lists p-values for individual pairwise phylum-level comparisons (SOM pools; Table S2).

L188 – was this significant, please present the statistical test.

Yes, this was significant. We added the appropriate p-values to the text (now L207).

192 – total SOM or Total C?

Thank you for catching this. "Total C" is correct, and we updated the text accordingly (L212).

L195 – PHOS seem to have a higher VIP value than phenol. Perhaps it should be included in the text?

Yes, PHOS had a slightly higher VIP than that of phenols in the PLSR model for chemically stable C. This means that across all of the latent factors the model created to describe variation in chemically stable C, PHOS was one of the most important variables. However, phenols had a slightly higher X loading score (compared with PHOS) on the most explanatory latent factor describing chemically stable C. Because X loading scores indicate which latent factors describe the greatest amount of variation in the response variable, they are typically the focus of PLSR model interpretation.

Because of the high number of fungal traits that we characterized in this study, there are certainly additional relationships that we could highlight in the text. In this section of the Results, we chose to focus on the traits that emerged as important for either total C and MAOM, or chemically stable and biologically stable C. PHOS was an important predictor of chemically stable C, but not as important for biologically stable C; in contrast, biomass phenol content was an important predictor of both biologically and chemically stable C pools.

L201 – There is no mention to WSA. Please add information on this.

As requested, we added a sentence about the water-stable aggregate (WSA) results to this section (L221-222 of revised manuscript).

L208 – the relationships described here are not linear, what function did the authors fit, was it a quadratic function? Can the authors claim that PC1 is associated with greater chemically and biologically stable C in a parabola? Is this due to differences between Ascomycota and Mucoromycotina? Please be precise and specific and add enough detail and be cautious in your interpretations.

In the Methods section, we describe the use of polynomial regression, fitting the model with the lowest mean square error between each predictor and response variable. In this case, the polynomial regression model indicated a two-degree model should be used (i.e., quadratic model). We added text to this section to specify that we were describing the results of a quadratic polynomial regression model (L229). We have observed that it is common in the literature to use terminology such as "generally associated with [greater/lower] values…" to describe the relationship between predictor and response variables in quadratic polynomial models, like the ones we present in Fig. S6.

L212 – what do the authors mean with intermediate performance, please describe specifically, and refer to figures and statistical tests. From PCA and PLSR it is difficult to draw such conclusions. And I also question what this subjective term means.

Please see previous responses to your questions about the meaning of "intermediate values" in the context of our study. We hope that the revisions we made above have clarified that we are referring to trait values that fall within the middle of the range of values exhibited by the eight fungal isolates.

For clarity, we revised the text in this section to address this reviewer's comment about PCA and PLSR. The text now refers more broadly to the suite of analyses that we presented in Figs. 1-4 and reads (L232): "The results of these analyses (Fig. 1-4) led us to hypothesize that microbial taxa with intermediate to high performance across this key grouping of traits would be most proficient at…". The PCA and PLSR analyses allowed us to identify clusters of traits that were associated with the formation of different SOM pools. Looking across all our results, we noticed that some taxa made larger contributions to a broader range of SOM pools, whereas other taxa were only proficient at generating SOM within a couple of the measured pools. Thus, our intention here is to describe how a new hypothesis emerged during our analysis of the data, leading us to calculate metrics of trait multifunctionality and SOM formation potential.

L217-218 – is the hypothesis in L211-214 accepted or rejected?

The hypothesis was supported. This is illustrated through our description of the results, and the examples that are presented at L242-249 (revised version).

L224 – Refer to the figure where this data is presented.

We added a reference to Fig. 3 (L246 in revised manuscript).

L226 – what is an intermediate CUE? How were these categories defined and in comparison to what is this intermediate?

Please see previous comments.

L230 – what figure here?

We added a reference to Fig. 1 (L251).

L234 – what is fungal identity? Do the authors mean whether there are differences between species in terms of their effect on SOM chemistry? I suggest using clearer and more precise language. Please rephrase for clarity.

As suggested, we rephrased this sentence for clarity. It now reads: "…we sought to understand whether species identity influenced SOM chemistry" (L255).

L255 – does this mean that if there is a higher number of chemical compounds the proportion of chemically stable C is higher? How is this explained versus the perhaps strong contribution of a few compounds?

This sentence (now L275-278) refers to a metric of SOM chemical diversity based on the Shannon index, which in this context accounts for both the richness (number of distinct compounds) and evenness (relative abundances of each compound) of SOM. In the Results, we report that we observed a positive correlation between SOM chemical diversity and the proportion of chemically stable C formed by fungal isolates. This is a correlational analysis, and readers can assess the relative strength of this relationship alongside the relationship between proteins (or phenols) and chemically stable C in Fig. 6b-c.

L260 – Did your estimates of CUE make sense? How do they compare to other studies? For instance, CUE of 0.8 is often considered the theoretical maximum. Please provide some context to your findings.

As requested, we added a discussion of the CUE results to the Supplementary Materials. We compare our measured CUE values (across the three methods that were employed in our study) to values reported in the literature. We incorporated this information into a broader discussion of the three methods for measuring CUE, which we added to the new Supplementary Methods section ("Key experimental design decisions & rationale").

L265 – how did respiration look like? Please present the respiration data.

We added a new supplementary figure showing the respiration data for each fungal isolate (Fig. S3).

L265-266 – so is it species with high multifunctionality or with intermediate performance? This is rather confusing. And what species were these?

It is both. The sentence reads: "Species with higher trait multifunctionality, especially those with intermediate to high performance across this key grouping of traits…" implying that there was a subset of fungal species that had high trait multifunctionality scores and intermediate (to high) values across the cluster of traits identified as important for SOM formation. As discussed in the paragraph beginning at L312 (revised version), isolates that invest at intermediate levels across a wider range of trait categories may be more likely to have the genetic and/or physiological

capacity for traits important to SOM formation, whereas specialized species may be more likely to lack the genetic or physiological capacity to perform such relevant functions.

L273 – aggregate formation is not mentioned in the results section, please add.

The analysis of water-stable aggregates is referenced in the results section at L192 and L202, and these results are presented in Figs. 3 and 4. We added an additional sentence describing results related to water-stable aggregate formation at L221 in the revised manuscript.

L290 – what were the traits that contributed the most to SOM formation? Have similar results been found by other studies? Please provide context.

L290 of the original manuscript (L311 of revised version) is a transition sentence, where we indicate that we will be discussing relationships between fungal traits and SOM formation in the next paragraph. At L312, we begin with a discussion of trait multifunctionality. We describe the specific relationships observed between individual fungal traits and SOM pools beginning in the following paragraph (at L333). In each of these sections, we discuss how our results compare and contrast to prior studies, with appropriate references.

L300 – CUE was, in the PLSR model, one of the most important traits contributing to SOM formation. How is CUE related to being a generalist and having intermediate trait multifunctionality? Can we learn something about physiological and genetic trade-offs? I suggest the following read:
Saifuddin, M., et al. (2019). "Microbial carbon use efficiency predicted from genome-scale metabolic models." Nature Communications 10.

This is an interesting question. The study by Saifuddin et al. (2019) demonstrated a negative correlation between bacterial CUE and genome size, finding that taxa with larger genomes had the genetic capacity for access and uptake of a broader range of substrates. The results of their study may suggest that there is a genetic trade-off between CUE and the performance of other traits, such as the production of specific enzymes. These results do appear to align with our findings that taxa with intermediate CUE values exhibited a greater potential for performance (intermediate to high trait values) of other traits important for the formation of SOM. We considered adding a statement about the study by Saifuddin et al. (2019) as it related to our results; however, the focus of our study is on the relationships between fungal trait profiles and the formation of different SOM pools. Based on other comments from the three reviewers, we took extra care to avoid discussions that may imply our results demonstrate innate or immutable characteristics of the fungal isolates studied in this experiment (e.g., genetic tradeoffs exhibited by particular species). Therefore, we decided not to add this comparison to the Saifuddin et al. (2019) study.

L303 – What is meant by “bundles of functions with synergies”? Please use clearer terms and avoid the use of subjective expressions in quotation marks.

This is a direct quotation from Fiedler et al. 2021 (ref. #60), which is provided as a citation immediately after the quote (L323 of revised manuscript). We use this exact term in quotation

marks because we felt that the precise wording used by Fiedler et al. expressed a novel ecological concept that was directly relevant to a discussion of our results. We have opted to keep this quotation in the manuscript to give credit to Fiedler et al. (2021), and because we feel that the use of quotation can be an effective way to place emphasis on important concepts and terminology.

L307 – can the authors clarify what is a "single community level syndrome"?

For clarity, we removed "single" from the text, such that it now reads "community-level syndrome" (L328). This is followed by examples of some of these proposed syndromes (e.g., "high yield" vs. "resource acquisition"; *sensu* Malik et al., 2019).

L321 – can you mention a couple of reasons why intermediate SOM formation would amplify SOM formation potential?

Intermediate to high fungal contributions to multiple SOM pools amplified SOM formation potential because the calculation of SOM formation potential was based on average values for each SOM pool as well as a metric akin to evenness (i.e., relative contributions to SOM pools across the suite of SOM pools measured in the study). That said, L321 in the original submission (now L342) discussed the patterns we observed between intermediate to high *trait values* and SOM formation potential, and thus, we were unsure if "intermediate SOM formation" in the reviewer's comment above was a typo. We present possible explanations for these relationships throughout the next 4-5 paragraphs of the Discussion. We have also addressed this reviewer's previous comments about the meaning of "intermediate" trait values and we hope these responses have helped to address this specific question as well.

L317-326 - How did respiration rates looked like? Can that explain some of these findings?

We added respiration data to the supplementary materials (Fig. S3), such that readers can assess these results alongside our findings about CUE and growth rate relationships to SOM formation. While there were differences in respiration rates across isolates, we standardized the length of incubation experiments based on the growth dynamics of each isolate (described in detail in Methods). The section that the reviewer asked about discusses relationships between fungal traits and relatively stable pools of SOM (e.g., biologically and chemically stable C). Fungal traits exhibited distinct relationships with total C compared to biologically/chemically stable C pools (Fig. 4), suggesting that differences in total respiration alone cannot explain an isolate's contributions to persistent pools of SOM. Our results suggest that higher growth rates and an abundance of certain biomass compounds (proteins, phenols) were important positive predictors of contributions to persistent pools of SOM.

L337 – what is meant by activity levels? Is it growth? Respiration? Please be specific.

We intentionally used the term "activity levels" here to refer broadly to processes associated with active microbial communities, which could include high levels of enzyme production and decomposition, metabolite and EPS production, biomass production, and more. The latter part of this sentence and paragraph (L358-360 revised manuscript) describes higher biomass turnover,

enzyme production and processing/decomposition of necromass as examples of such activities. Therefore, we chose to leave the broad language of "activity levels" in this section of the manuscript.

L344 – how would this be different from high CUE with high growth and low respiration?

This sentence reads "In contrast, if fungi are efficient (i.e., high CUE) but their growth rate is slow…" (now L365). This is different from the scenario that the reviewer describes because we are referring to fungi with high CUE and a *low* growth rate. A fungus could have a high CUE and a relatively slow growth rate if its respiration rate is also proportionally low.

L346 – what is meant by "field soils"? environmental soil samples versus model soils?

Yes, "environmental soil samples" is another possible way to word this. We have commonly seen "field soils" or "natural soils" used in related literature. For example, a prior study that examined microbial formation of SOM in model soils described the natural environmental soil sample as the "field soil" reference sample (Kallenbach et al., 2016, *Nature Communications*). We have therefore opted to keep the terminology "field soils," as we think this term is consistent with past published studies in our subfield.

L351 – what is the size of this fraction, was it quantified?

Yes, this is a reference to the pools of biologically stable C and chemically stable C that were quantified (e.g., Fig. 3). We reworded this sentence for clarity as follows: "however, only a small fraction of this C was biologically or chemically stable" (L371).

L354 – does more active fungal species mean intermediate? The choice of words is vague. More active in comparison to what?

As described above, we used the term "more active" to refer broadly to active microbial communities that are often characterized by elevated rates of activity for multiple processes, including growth rates, enzyme activities, metabolite production, and more. The sentence that the reviewer refers to lists the specific traits (growth rates, CUE, turnover rates, enzyme activities) for which we observed an association between intermediate/high trait values and SOM formation (L375-378).

L392 – This reference is also context dependant. Could the authors provide what the differences are in other contexts with references?

Reviewer 3 also commented on this. "Context dependent" in this case referred to the divergent relationships observed among microbial traits and different SOM pools, as demonstrated in our study and others (e.g., Craig et al., 2022, *Nature Communications*). We revised the first two sentences of this paragraph to specify the context dependent relationships we were referring to (L412-413 of revised manuscript).

L 394 – Did the authors find any no-multifunctional microbes? If not, then this sentence needs to be re-written.

We revised this sentence to say "…is promoted by more multifunctional microbes" (L414) to clarify that we are referring to those taxa that had higher multifunctionality scores/values among the eight fungal isolates that were studied.

L 402 – But wasn't CUE the most important contributor to SOM formation? So why intermediate?

At L415 (revised manuscript), we clarified that we are referring to taxa with intermediate *to high* trait values for the particular grouping of traits that we identified as important for SOM formation. CUE was the most important trait contributing to variation in total C and MAOM-C, but not to biologically or chemically stable C. As we have emphasized elsewhere in the Discussion, our findings suggest that multiple fungal/microbial traits are important for the formation of functionally complex SOM (including total C, MAOM and more stable SOM subfractions). Thus, we describe how species with intermediate to high performance of CUE alongside other key traits (e.g., growth rate, biomass protein and phenol contents) made the largest contributions to multiple SOM pools (i.e., highest SOM formation potentials).

L 403-406 – does the data support this statement? Please provide details with mentions to figures.

This question refers to a sentence in the concluding paragraph of the Discussion section (now L423-426). In our experience, it is not common to reference figures in the Discussion, especially in a concluding paragraph. However, the statement that the reviewer refers to is supported by our results. Below, we detail the supporting sections of the Discussion that support this statement:

"Specifically, we demonstrate that CUE alone is inadequate to predict the formation of SOM across all functional pools…"

- CUE was an important positive predictor of total C and MAOM-C, but did not explain a significant amount of variation in biologically stable C or chemically stable C. Other variables (e.g., growth rate, biomass protein and phenol contents, etc.) were more important positive predictors of these SOM pools. For example, see discussion at L333-342.

"…and may be decoupled from SOM stabilization when 'formation' traits (e.g., biomass protein and phenol contents) influence the subset of microbial residues that are incorporated into stable SOM pools."

- Here, we suggest that our results may indicate that CUE appears decoupled from the formation of relatively stable SOM pools because other fungal/microbial traits influence the formation of these pools. For example, see discussion at L351-364. We intentionally use the phrase "may be" to emphasize that we are suggesting a possible interpretation of our results.

L412 – I think that the use of a model soil and the measurements in this manuscript should be carefully described as accounting for pathways by which microbial residues are incorporated into SOM pools. I would like the authors to comment on this.

In response to a comment from Reviewer 3, we revised this concluding section to remove the reference to "pathways by which microbial residues are incorporated into SOM." It now reads (L430-433): "Our results demonstrate the importance of accounting for the formation of multiple SOM pools and the synergies among microbial traits that promote their formation. Future research would benefit from a more comprehensive approach that can account for these complexities, given divergent relationships between specific microbial traits and SOM pools." We feel that this revised version is a more specific and accurate statement of the key take-aways from our experiment.

Methods

In the Guide for authors it is clearly stated that "for all statistics (including error bars), provide the EXACT n values used to calculate the statistics (reporting individual values rather than a range if n varied among experiments)." I don't think the authors have reported this. Please check and add the information.

Thank you for this suggestion. We had reported values for n in the original version within the Methods section text. To improve clarity, we added these values to figure captions as well in the revised manuscript.

L458 – was this a one-time addition?

No, there were three total substrate additions. This is described shortly after this line at L490 (revised manuscript).

L459 – is this soil CN ratio similar to that of Harvard forest?

The C:N ratio is similar to those observed at Harvard Forest (which range from ~16-27 for mineral soils; Compton & Boone; 2000). We chose a C:N ratio of 20:1 specifically as it was expected to alleviate N limitation for fungal isolates (whose average biomass C:N ratio was ~10) (Manzoni et al., 2012; Sinsabaugh et al., 2016). We added this rationale to the new supplementary methods section ("Key experimental design decisions & rationale").

L472 – is the choice of 840 mg C based on a published study, or how was this calculated?

We added a rationale for this level of C addition to the new supplementary methods section ("Key experimental design decisions & rationale").

L 473 – where is the Respiration data? I cannot find it in the main manuscript or supplementary material. This data is important because it balances growth in CUE. The authors refer in the

results section to higher or lower growth but do not mention respiration. Please provide the data and include it in your results and discussion where useful.

Respiration data have been added to the supplement (Fig. S3).

L478 – what is this choice of respiration rate based on?

In this experiment, respiration < 1 ug CO_2-C g^{-1} soil h^{-1} represented a near-zero respiration rate. We added respiration data to the supplementary materials (Fig. S3), which provides needed context for understanding that this was a low value relative to peak respiration values observed following substrate additions. We provided additional explanation and rationale for this decision in the new supplementary methods section that we added ("Key experimental design decisions & rationale").

L485 – how many replicates were included exactly?

A total of eight replicates were established for each isolate to ensure that sufficient replication would remain in case of contamination. The species that was slowest to establish during initial growth and biomass production, *P. stipticus*, was the most challenging taxa to avoid contamination. All other species had at least four remaining replicates that were free of contamination, with most species having 5-8 replicates remaining. These details have been added to the new supplementary methods section.

L523 – how was respiration measured here?

The LI-COR method (described earlier in the Methods section) was also used to measure respiration here. We added that detail to the text (L558 of revised manuscript).

L595 – what is "(5)"? and what is the conversion factor used? Please include that in the text.

The published conversion factor that was used is 5.0, as proposed by Anderson and Martens (2013) (reference #78). To clarify this point, we revised the sentence as follows (L564): "Microbial biomass C (MBC) was estimated from DNA concentrations using a published conversion factor of 5.0 to convert μg DNA g^{-1} soil to μg MBC g^{-1} soil[78]."

L536 – Can you explain in detail how is respiration obtained by the slope?

We plotted respiration rate (μg CO_2-C produced g^{-1} soil) against time (hours) for each isolate, where the slope of the linear trendline represented the rate of change in respiration (y) over time (x). For each isolate, we performed this regression for each of the replicates, and the average slope of these lines was calculated to acquire the average respiration rate values that were included in subsequent calculations of carbon use efficiency. Based on a comment from Reviewer 1, we more clearly described how this approach (based on linear regression and associated slope values) was used to estimate growth rates (L568-570). The next sentence discusses how this same approach was used to estimate respiration rates and to calculate CUE for each isolate. We hope this clarifies our approach.

L537 – I would like the author to explain the use of standard error in a model soil with 8 fungal isolates since the standard error measures how far the sample mean (average) of the data is likely to be from the true population mean. What would your true population be and how representative are your experimental systems to a true population?

In the context of our study, the true population mean is the true average value (e.g., trait value, SOM pool size value, etc.) for an individual isolate growing under our experimental conditions. We included 3-4 replicates per isolate in the statistical analyses conducted in this study. If we had included more replicates, the standard error of the mean (SEM) would get smaller as we got closer to the true mean of the population. Thus, SEM reflects the standard deviation (variation around the mean of the replicates) and error (certainty/uncertainty) that the average value determined by any given study represents the true mean value of the statistical population. We do not intend to imply that it represents the true mean of the *biological population* for any given isolate. Our study was focused on understanding relationships between relative trait values (across isolates) and the formation of different SOM pools. As addressed below in response to a comment by Reviewer 3, we added two sentences early in the manuscript to ensure that this focus is clear (L154-160).

L 565 – is this mentioned in the discussion, or are these results presented and discussed without mention?

We had originally included this text in the Discussion section but moved it to the Methods section based on word limits for the Discussion. The results of the biomass turnover assays did not figure heavily into our central discussion and conclusions. The isolate for which biomass turnover rate may be artificially inflated (*Gymnopus sp.)* actually had a relatively low SOM formation potential relative to the other isolates; thus, the pattern we observed between turnover rates and SOM formation potential would actually be strengthened if the biomass turnover value for *Gymnopus sp.* was lower. We chose to leave this text in the Methods section (currently 599) rather than include this lengthy explanation in the Discussion.

L686 – what R package was used for PLSR?

PLSR was conducted in JMP. This was stated in L689-690 of the original manuscript, which is now L733: “PLSR was conducted in JMP Pro (version 16.1.0) using the NIPALS algorithm (as in[96]).”

L 698 – add that VIP stands for Variable Importance in Projection, this is present in the Figure 4 caption but should be in the main text too. Also, VIPs are often used as variable selection, and this involves setting a VIP value threshold. What value did the authors choose for variable selection?

We added that VIP stands for “variable importance in projection” at L742. We used the common threshold of VIP > 0.8 to determine which predictor variables to include in the final models (e.g., as in Smith et al., 2018 [ref. #96]). This detail has also been added to the text (L743).

Reviewer #3 (Remarks to the Author):

The manuscript of Whalen et al. presents an interesting approach to derive insights on the interactions of microbial traits with soil organic matter transformation. The authors used a simple model system with sand grains and montmorillonite particles incubated axenically with various fungal species isolated from a hardwood forest. Glucose and potato infusion extract were used to provide C and ammonium nitrate to provide N. The substrate was added three times at 5.6 mg C g-1 amounting to 16.8 mg C g-1 over the course of 3-6 months. The authors evaluated various fungal traits and properties of the remaining model soil to evaluate the fungal-derived organic residues. A multifunctionality indicator is computed and correlated with the residue properties. The manuscript provides novel detailed insights that promote the understanding of soil organic matter dynamics.

We are very glad to hear that this reviewer found our paper to provide "novel detailed insights that promote the understanding of soil organic matter dynamics."

However, various major aspects should be considered:

• The performance of individual fungi isolated form a hardwood forest to utilize glucose does not represent the transformation of more complex plant litter, since the fungal species originate from an ecosystem where specialized traits to decompose litter derived from the hardwood vegetation and the ability to cooperate are more pronounced. The limitations of using glucose as a model substrate are not adequately addressed (e.g. l. 348). Could the fungi that turned out to perform as multifunctionalists in this manuscript (l. 295) simply be more specialized in glucose consumption? It is questionable to which extent insights on species-specific organic matter transformation (l. 348) can be inferred since the adaptation to glucose is reported to suppress the enzymatic activity for the assimilation of other C sources.

Glucose has been used in numerous studies as a model substrate and has been shown to be rapidly assimilated and metabolized by a wide range of microbial taxa (Hill et al., 2008), including saprotrophic fungi (Rousk & Baath, 2011). As described in our response to Reviewer 1, we strategically designed the timing of our experimental substrate additions and harvests to promote near-complete utilization of substrate-C. Compared to other C-supplying substrates, glucose exhibits low levels of discrimination among microbial taxa (Jones et al., 2018), and there is robust support for its use in incubation experiments as a C source for microbial/fungal growth (e.g., Kallenbach et al., 2016; Jones et al., 2018). We added a sentence to the methods section (L481-484) providing this rationale and directing readers to a more detailed discussion in the supplement (within 'Key experimental design decisions & rationale').

We agree that Reviewer 3 raises important concerns about the ability of glucose as a model substrate to represent species' capacity for the formation of SOM. Indeed, given a more complex substrate, certain species (e.g., slow-growing Basidiomycota) may have been capable of greater SOM formation. However, our focus in this manuscript was on relative differences across fungal trait profiles, rather than describing innate or immutable characteristics or capabilities (e.g., SOM formation potential) of the fungal isolates themselves. By using a group of eight fungal isolates,

our goal was to span a gradient of trait values across a suite of fungal traits, such that we could evaluate relationships between fungal trait profiles and SOM formation. In response to this reviewer's comment, we added explicit discussion of these aims early in the manuscript at L154-160. Additionally, we revised specific language throughout the manuscript to clarify these aims. For example, in the original draft we had used the term "capacity" or "ability" several times, referring to an isolate's capacity to perform a trait. While we only meant to refer to an isolate's capacity to perform a certain trait under the experimental conditions of our study, we decided to replace these terms in the revised version to avoid confusion. Specific line edits were made at the end of the introduction (e.g., removed mention of "fungal capacities" at L134, original version), L151-154, L191, and the first paragraph of the discussion (L281).

• The data seems to indicate that the recycling of the fungi's own biomass, as seen for example by the increased phosphatase enzymatic activity, plays an important role (l. 230, l. 341). It is not clear whether this observation is affected by the variation of incubation times ranging from 3 to 6 months. The necromass/biomass recycling affects the evaluation of the carbon use efficiency, which describes the partitioning of carbon for biomass growth versus respiration. Two different approaches were used in the manuscript to evaluate the CUE and it is not clear which one the text is referring to when mentioned in the results (l. 192). This aspect is especially important to clarify since the manuscript claims to demonstrate that "CUE alone is inadequate to predict the formation of SOM" (l. 403).

The reviewer makes two important points here. First, our data did suggest that biomass recycling may have played an important role in SOM formation, as indicated by the relationship between phosphatase (PHOS) activity and chemically/biologically stable C. The reviewer asked whether this observation could be affected by the variation of incubation times ranging from 3-6 months. We interpret this as a question about whether the isolates that were incubated for a longer period of time (6 months) may have shown greater biomass recycling simply because of this longer incubation period. However, we observed the opposite trend—the faster-growing isolates that were incubated for closer to three months demonstrated a greater potential for PHOS activity, and we discuss that this might be related to greater biomass recycling among these taxa (e.g., L361-364).

The reviewer also makes the point that CUE values are affected by necromass/biomass recycling. While this is true, we included three different metrics of CUE to address this potential concern. Two of the metrics were derived from short-term incubations (either in soil or liquid culture) that were designed to measure an isolate's CUE during log-phase growth, before biomass recycling begins (Geyer et al., 2019). The third metric of CUE was evaluated during stationary growth (using the ^{18}O method), such that biomass turnover rates could also be assessed. The reviewer's question about the result stated at L192 refers to the PLSR results presented in Figure 4a. In this figure, we present the loading scores and VIP values separately for each of the three CUE metrics, showing their relative importance as predictors of each SOM pool. We concluded that CUE was the "most important trait regulating the formation of MAOM and total SOM" because the three CUE metrics had some of the strongest positive loading scores and highest VIP values of any of the fungal traits included in the PLSR models for these two SOM pools. To avoid potential confusion, we reworded this sentence to say that CUE was "one of the most important traits" regulating the formation of these two SOM pools (L212). Additionally, we added further

discussion and rationale for the inclusion of these three metrics of CUE to the new supplementary methods section ("Key experimental design decisions & rationale").

• It should be noted that bacteria were not included at all, i.e. "understanding the role of microbial traits" (l. 267) is limited to fungal traits only.

As suggested, we replaced "microbial" with "fungal" (L288 of revised manuscript).

• The production of different fungal-derived protein and phenol contents is quite interesting. It might be advisable to strengthen the conclusions on this part, e.g. extend the finding that "the source of many soil phenols is unknown" (l. 372). However, here it is advisable to consider older literature, which was strongly focused on phenols form plant or microbial, and especially fungal origin as precursors in SOM formation.

We are glad to hear that this reviewer found our insights on microbial-derived phenols in SOM to be interesting and novel. As suggested, we edited the text in this section to further emphasize the discussion around microbial-derived phenols. However, due to journal word limits, we opted to keep this discussion brief by: (1) adding additional citations of studies that have demonstrated microbial/fungal production of phenols in soils (Kallenbach et al., 2016; Wang et al., 2017 [refs. 14 & 57], and (2) pointing readers towards our recent publication that has a longer discussion on this topic (Whalen et al. 2022 [ref. #6]). These edits are reflected in L393-395.

• The context dependency (l. 392) and the modulation of trait activity by soil properties (l. 389) are not clear.

"Context dependent" in this case referred to the divergent relationships observed among microbial traits and different SOM pools, as demonstrated in our study and others (e.g., Craig et al., 2022, *Nature Communications*). We revised the first two sentences of this paragraph to specify the context dependent relationships we were referring to (L412-413 of revised manuscript).

To improve clarity, we rephrased the sentence that had used the phrase "modulation of trait activity by soil properties." We removed this aspect of the discussion, focusing instead on the core point that microbes may play a role in modulating the emergence of SOM functional complexity in soils. The sentence now reads (L409-411): "Thus, microbial communities and their associated traits may play a role in the emergence of SOM functional complexity in soils, as is recognized for other characteristics of the soil habitat (Phillipot et al., 2024)."

• There does not seem to be a clear pattern related to MOM vs. POM formation of fungal residues. (Fig. 3). It is necessary to report the recovery of C and N in the fractions of their bulk contents (l. 603).

We updated Fig. S3 (now Fig. S4) from the original submission to include (1) mg MAOM-C g^{-1} soil and mg POM-C g^{-1} soil, and (2) the proportion of total C that was present as MAOM-C or POM-C. This figure illustrates the pattern that the reviewer was referring to.

• It is necessary to come up with clear messages derived from the incubation experiments, rather than quite non-descript claims for a more "holistic approach". This is especially necessary, as "pathways by which microbial residues are incorporated into different SOM pools" have been studied among others with isotopic approaches are extensively reported in the literature.

Thank you for this suggestion. We revised the concluding sentence of this paragraph as follows to avoid the potential confusion/issue raised by this reviewer (L430-433):

"Our results demonstrate the importance of accounting for the formation of multiple SOM pools and the synergies among microbial traits that promote their formation. Future research would benefit from a more comprehensive approach that can account for these complexities, given divergent relationships between specific microbial traits and SOM pools."

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank Reviewer 4 for taking the time to provide this co-review.

REVIEWER COMMENTS

Reviewer #1 (Remarks to the Author):

The authors significantly revised the manuscript, addressing all Reviewer comments thoroughly. I have few comments on the revised Manuscript, and one more comment addressing one of the answers. I am sure the authors can integrate these suggestions, and I strongly encourage Publication of the study in Nature Communications.
I thank the authors for their thoughtful answer to my comments on glucose leftovers influencing the results. Looking at Fig. S3, I agree that it seems likely that glucose was used and respired completely by all fungal isolates in this experimental design. I still find it an important point, since it cannot be completely ruled out that there are leftovers of medium components, and this point must be carefully considered in future designs. In fact, I have already seen and been involved in experiments planned with a similar design, and depending on conditions and research questions glucose may be problematic, as well as other medium components like proteins. In case there is another limiting factor to growth, e.g., an environmental stressor that may limit fungal growth and/or another element limitation in certain isolates by N, P or even micronutrients. For this reason, I find it important to mention such potential biases in the main text, not only in the Supplements. This point should be clearly discussed either in the Methods or Discussion sections.

Thank you for this suggestion. As requested, we added the following text to the Methods section at L512-519: "A central concern was ensuring that most of the added substrate media was utilized by fungi prior to incubation harvest; had all species been incubated for the same amount of time, it is more likely that unprocessed substrate would have remained in soils for slower-growing species compared with faster-growing species. While it cannot be ruled out that a small fraction of media components remained in soils – especially if fungi experienced nutrient (e.g., N, P) limitation or environmental stress (e.g., space limitation) – our approach to standardization minimized the potential for such biases across fungal taxa (additional rationale provided in supplementary materials)."

Supplementary Material:

L99: It may also be an option to determine the amount of C lossed via CO2? There are likely lower and upper limits to CUE of fungi, so the C lossed should be relatively comparable among isolates in case all isolates used glucose. Though I agree, there will be different types of recycling of material and high variation in efficiencies. But it may be a good estimate whether values are comparable over time.

We agree that determining the amount of C lost from soils as CO_2 (respiration) is informative. At L100 in the supplementary materials, the second approach we suggest is measurement of total fungal biomass alongside an assessment of the total amount of C respired as CO_2 throughout the course of the incubation experiment. Because CUE and biomass turnover/recycling rates varied across taxa (as mentioned by the reviewer), measuring fungal biomass alongside cumulative CO_2 respired would provide a more accurate picture of total C utilization. To ensure it is clear that we

were talking about calculating the total amount of C respired as CO_2 by each fungal species, we revised the language at L101 of the supplementary materials.

L151-153 To put this text into context, it would be helpful to add the formulas and response variables used for different CUE estimates.

As requested, we added the formulas used to calculate each CUE metric at L153-155 of the supplementary materials.

Main Manuscript:

Fig. 4B It may be helpful to indicate in the x-axis the differences in the parameters. This way it looks like three plots are showing exactly the same

The X axes here are representative of the same series of CUE data; however, the data along the Y axis is what varies in this set of figures. We chose to include an overarching label ("SOM functional pool (%)") along the Y axis such that the scatter plots for each SOM pool would be aligned with/underneath the corresponding plots representing the PLSR results for each SOM pool. We describe this in detail in the caption for Figure 4(b). In panel (b), the first scatter plot represents the regression between CUE and total C. The second panel represents the regression between CUE and MAOM-C, and so on. We revised the text in the figure caption to ensure this is clear.

Fig. 4A Maybe I missed it, what is the unit of chem- and bio-stable C; is it a proportion?

The unit for chemically and biologically stable C is a percent (%); that is, the proportion of remaining soil C that resisted chemical or biological destabilization in the two assays that we conducted to determine relative fungal contributions to persistent pools of SOM. This is indicated in Fig. 3 (y axis labels), which shows fungal contributions to each of the measured SOM pools, including chemically and biologically stable C pools (%). These assays and the fraction/portion of SOM represented by "chemically stable C" and "biologically stable C" are also described in the Methods at L687-690 and 711-713.

Reviewer #2 (Remarks to the Author):

General comments:
The present manuscript aimed at describing microbial traits that predict soil organic matter (SOM) formation. The authors used model soils and 8 fungal strains to quantify in the lab several physiological parameters such as Carbon Use efficiency (CUE), fungal growth, enzyme production, along with the formation and stability of soil particles. In addition, the authors assess how the degree of microbial trait multifunctionality affects SOM formation and stability. This is

an interesting, relevant, lab-intensive, and ambitious approach. One of the most interesting finding is that fungal species with the highest SOM formation potential are also responsible for generating more chemically diverse and stable SOM. The strength of this manuscript is that it provides and relates, using adequate analysis, multiple measurements that support the abovementioned findings.

We are very grateful to hear that Reviewer 2 finds our manuscript to be "interesting, relevant, lab-intensive and ambitious."

However, after reading the manuscript again I believe it has some issues, not in terms of methodology but in the ways it moves the field forward. The study lacks context in field soils, and the authors do not succeed in comparing their findings to the multitude of papers on SOM formation that are published. In addition, the significance of the study to the field is limited by the impossibility to generalize their results without a field trial. This study needs to be complemented with a field study exploring how trait multifunctionality can explain soil formation and stabilization in natural environments.

We are very glad to hear that Reviewer 2 found our methodology to be robust. While a field trial would certainly complement and strengthen our lab-based experiment, it was not within the scope of this project. Many lab-based experiments have significantly advanced our understanding of SOM formation processes and/or relationships between microbial community characteristics and SOM accrual (e.g., Golchin et al., 1996; Sanderman et al., 2014; Kallenbach et al., 2016; Domeignoz-Horta et al., 2020, 2022; all cited in our reference section). While we did consider conducting a field-based trial, several limitations informed our decision towards a lab-based experiment, including (1) it is challenging to link microbial species identity, traits, and contributions to SOM under field conditions because the active and whole microbial communities that formed SOM throughout the experimental duration may not be reflected by the snapshot of community composition/activity captured by DNA/RNA extractions and other molecular assays on field soils. Second (2), it is more challenging to control for experimental conditions in the field than in the lab, introducing additional sources of variation into the dataset. Because changes in SOM pools can be challenging to detect over short time periods, we ultimately decided that the controlled conditions of a lab experiment would reduce Type II error and maximize our chances of detecting differences in fungal species' contributions to SOM quantity, stability and chemistry, should such differences exist. While a field study is therefore outside the scope of this manuscript, we agree that field-based studies would be an excellent direction for future research. Our manuscript and central findings offer a framework for future field-based work linking microbial traits to SOM formation processes, highlighting the importance of considering multiple microbial traits (multifunctionality) alongside measurements of multiple SOM pools (e.g., total C, MAOM-C, POM-C, etc.).

Related to the reviewer's comment about references, we aimed to compare our findings with the most relevant papers in the literature, but due to a limitation on the number of references, we could not highlight them all. We have referenced several review papers that comprehensively examined SOM formation (e.g., Oades, 1984; Baldock & Skjemstad, 2000; Schmidt et al., 2011; Lehmann & Kleber, 2015; Kleber et al., 2015, 2021; Lavallee et al., 2020; Angst et al., 2021; Sokol et al., 2022; Whalen et al., 2022), such that our reference list encompasses both historical

and contemporary literature on SOM formation dynamics. Additionally, in this revision (#2) we added a reference to an important early manuscript that demonstrated microbial formation of chemically complex SOM in a model soil system (Golchin et al., 1996; replaced Fiedler et al. 2021 reference that was removed in response to another comment below).

The manuscript still includes too much jargon and terms in brackets that do not add anything to the text. The discussion is superficial, lacks structure and clarity. The introduction is vague and the authors present one hypothesis which is a very unspecific research question. The unnecessary jargon and terms in brackets need to be removed (see specific comments), and clearer text will improve the readability.
Also, abbreviations throughout the text need to be checked.

We address this reviewer's specific comments below about "jargon, terms in brackets and abbreviations," removing certain terminology. Additionally, we do our best to respond to their concerns that the writing content was too "vague" in the Introduction and Discussion. We tailored our writing style/content to the broad audience of *Nature Communications*, as suggested by the Guidelines for Authors. This did necessarily make our framing of the topic in the Introduction broader than what is typically found in discipline-specific journals. We used our expert judgment to introduce and discuss what we saw as the most essential framing and discussion points with this broader audience in mind, also accounting for the word count limits on each section.

Please add units in the axis of each figure, same for tables, and not in captions, it makes interpretation more difficult. Figures need improvement as most results are presented by species and the results presented by phylum, and the color coding used does not work.

We have reviewed each figure and ensured that axis units are included where possible (e.g., Fig. 3, Fig. 4b, Fig. 6b). For Figures 1 and 4, adding units to the figures would make the text illegible, as it would need to be downsized substantially; this is the reason we chose to list the units in the figure captions. For Fig. 5, the axes are "Trait multifunctionality" and "SOM formation potential." These are synthetic axes (common in multivariate analyses), calculated based on multiple trait values or SOM pools, all associated with different units; therefore, these axis labels are unitless. Similarly, Figure 2a and 6a are ordinations with synthetic NMDS axes.

We address the additional concern mentioned here (about species and phylum-level presentation of results) below in the line comments section.

Specific comments
Line 64 - replace soil organic matter with SOM, already introduced in l49. Check throughout the manuscript

Thank you for pointing this out. We spelled out "soil organic matter" at L64 since it is common convention to spell-out abbreviations at the beginning of a sentence, and this appears to be the style preference for *Nature Communications*. Therefore, we left this particular instance of "Soil organic matter" at L64.

Line 93-98 – The commonly accepted explanation for the coupling between high CUE and SOM formation is because higher CUE entails higher allocation of resources to growth and biomass formation, and that microbial necromass, due to its chemical composition, remains in soil and thus contributes to SOM formation. The authors fail to explain this important link that I consider essential, and I suggest rephrasing because this is briefly mentioned in lines 128-129 but should be introduced earlier on.

Thank you for this suggestion. As suggested, we revised and added text at L92-95 expanding on this explanation for the relationship between CUE and SOM formation.

Line 108 – I have commented on this before and I am not convinced with the response given by the authors. The word "feedstock" is, as the authors replied, a terminology specifically introduced by Sokol et al. (2022) which has not been widely adopted. Sokol and his co-authors (which are authoring also this paper I am reviewing) will understand the term, and perhaps few others, but as mentioned, "feedstock" has not been widely adopted. The reason why I disagree with its use is because in an introduction the ideas/concepts are more important for the reader than the use of specific words. The authors are adding confusion and unnecessary jargon, and I would like to stress again that clarity of ideas is more important than the use of vague and unfamiliar terms. Same for "formation traits" in line 123.

As suggested, we removed and/or de-emphasized the terms "feedstock traits" and "MAOM formation traits" from the Introduction section. We rephrased these ideas in our own words and left only one reference to this conceptual trait framework in parentheses next to the Sokol et al. 2022 citation (ref. #18). These edits can be found at L109-112 and L123-124. Because this trait framework proposed by Sokol et al. in 2022 is still relatively new, it has not been widely adopted in the literature. As we describe in the introduction (L116-121), empirical tests of such a framework are currently lacking, and our aim is to integrate a comprehensive evaluation of microbial physiological, morphological and biochemical traits alongside measurements of SOM formation across multiple key functional pools (total SOM-C, MAOM-C, POM-C, water-stable aggregates, etc.). The lead author of the manuscript under review (E. Whalen) conceived of the concept for this framework during her Ph.D. work, and later expanded on it with Noah Sokol and colleagues in their *Functional Ecology* publication. The present study under review (one of E. Whalen's dissertation chapters) was developed with this framework in mind, recognizing that there may be distinct categories of microbial traits that regulate the initial production of microbial residues (later termed "feedstock traits") and those that influence the subsequent incorporation of microbial residues into stable SOM pools (termed "MAOM formation traits"). We hope that the revisions we made to our hypothesis section help to clarify this point. We left a single reference to this framework at L112 because we refer back to these concepts in the Discussion to provide potential explanations for patterns we observed among microbial traits and the formation of different SOM pools.

Line 129– there is no reference to why different fungal species were used, I think this should be included because the results are organized in that way, and this is stressed by the authors in lines155-156. Statistical results are presented for differences between phyla, so there must be a reason for this. It is unclear if there are expectations towards how different species contribute to

SOM formation differently, and whether there are specific hypothesis connected to different species/phyla? Please clarify.

Thank you for pointing this out. An earlier version of our manuscript stated this more clearly, and this text may have been removed (inadvertently) in our last round of edits. As suggested, we added this explanation back to the text at L130-132. The motivation for including these particular fungal species in our study was to have our "study system" (collection of fungal isolates) span a gradient of trait values. The focus of our study is on the relationships between fungal trait profiles and the formation of SOM (across multiple functional pools). Thus, we did not form particular hypotheses about inherent differences in SOM formation capacities of the specific fungal species (or phyla) included in our study, but rather on relative differences in their trait profiles. We hope the edits we made at L130-137 help to clarify this point, in addition to other edits we made in our last round of revisions (e.g., 156-161).

Line135-138 – the hypothesis is vague and the expected relationship between the variables and outcome is unclear. Please re-write in a concise and simple way, a hypothesis is a prediction and should be objective and testable.

As suggested, we rewrote our hypotheses (L138-144) to describe the specific predictions we had made about different categories of fungal traits and their relationships to the formation of different SOM pools. These details came from *a priori* hypotheses written by lead author Emily Whalen in her dissertation proposal. As discussed below in response to this reviewer's comment about L310-311, we did not include a hypothesis about fungal species grouping into "generalist" and "specialist" categories in terms of their trait functions as this was not an *a priori* hypothesis. This pattern emerged upon analyzing our data (*post hoc* observation).

Line 166– I think this needs to be toned down, I agree that Basidiomycota and Mucoromycotina are grouped and separated, but Ascomycota are quite spread along PC1 in Figure 2.

While it is true that the Ascomycota exhibited overlap with Basidiomycota samples along PC1, they are clearly differentiated from Basidiomycota along PC2, which was the second most explanatory PC axis. Additionally, there was no overlap in two-dimensional "species space" among fungal phyla within the NMDS ordination. Therefore, we feel that the statement, "Trait profiles exhibited clear groupings at the phylum level" is still an accurate description of these ordination results. Readers can then assess for themselves how the samples grouped by species and phyla in Fig. 2. Related to our responses below, it was not possible to describe the nuances of every figure at the species and phylum-levels in the text, given word limits on the Results and Discussion sections.

Line 166-184 – Throughout the manuscript it is difficult to follow Figures where measurements for each fungal species are presented but the text in the results section and some tables refer to fungal species grouped in Phyla. The color coding does not help as there is no explanation in the figure or caption. Please improve the figures and instead of color coding (or along) add what Phylum each species belong to.

As suggested, we added “Phyla” as a header to each of the plot legends/labels that were missing this information. The only exception is Fig. 1, as there was no elegant way to add these labels given the distribution of species within each phylum across the page. Because of word limits on the Results and Discussion sections, we found it necessary to summarize certain results at the phylum-level since there were only three fungal phyla, but eight fungal species. The high number of fungal species, trait measurements and SOM pools characterized made it necessary to simplify our written descriptions of the results in this way. However, we did not want to limit the information conveyed by each figure by only presenting results at the phylum-level; therefore, we included the full species-level datasets, where relevant, in figures.

Lines 167-168- by comparing different Phyla the authors do an ANOVA with groups with unequal sample sizes. Could this have affected the robustness of ANOVA and therefore your conclusions?

In our initial submission, we focused on species-level results to avoid this potential concern about unequal sample sizes. However, based on a request from Reviewer 2, we added the ANOVA results for the phylum-level comparisons (Tables S1 and S2). In the main text and supplement, we present ANOVA results at both the species- and phylum-levels to address this concern. Additionally, as we describe above, and in the manuscript (e.g., L159-165), the primary goal of our study is to evaluate relationships between fungal trait profiles and SOM formation. Therefore, our interpretations and conclusions focus on trait-SOM relationships, rather than on inherent or innate differences between SOM formation capacities of different fungal phyla. Because of this emphasis and our presentation of results at the species-level, we are confident that our conclusions are robust.

Line 231 – is GR introduced before? Please check.

Thank you for catching this. We spelled out “growth rate” here (L240).

Line 248-249 –In these lines the authors refer to the 5 fungal species with highest SOM formation potential stating that they are associated with higher SOM formation. It seems that this relationship doesn’t hold, because in Fig. 1 Gymnopus sp have high trait values for CUE and turnover but low SOM formation potential in Fig. S2B. I would like to hear the authors comment on this.

L248-249 (now L256-260) describes the trait values of the five fungal isolates with the highest SOM formation potentials. These results are presented to illustrate the overall positive correlation that we observed between trait multifunctionality and SOM formation potential. At L249, we describe the specific patterns we observed for *Gymnopus sp.*, which had a low trait multifunctionality score and correspondingly low SOM formation potential. Trait multifunctionality was calculated based on average trait values across the entire suite of measured traits. As described in the manuscript (e.g., L165-171), we observed that taxa with the highest trait multifunctionality scores (and SOM formation potentials) had intermediate to high values across a larger number of trait categories. In contrast, while *Gymnopus sp*. had high values for CUE and turnover, it had low trait values across a majority of the measured trait categories, contributing to a relatively low trait multifunctionality score compared to the other

fungal isolates included in this study. Thus, the results for *Gymnopus sp.* do support the overarching pattern that we describe.

Line 257 – the word "different" is missing before "fungal species"

As suggested, we added "different" before "fungal species" (L266).

Line 287-288 – It is widely accepted and that different microbial traits contribute to SOM formation and complexity. This paper adds empirical evidence that it is so, but it is an overstatement to say that the paper is "proposing that synergies among these traits promote SOM quantity, stability and functional complexity". Please remove this.

We are not entirely clear why this reviewer suggests that we remove this sentence. This statement (now L295-297) comes at the end of the first paragraph of the Discussion, where we summarize our key results and take-home messages. We agree that it is now widely accepted that microbial traits are important drivers of SOM formation and complexity; however, as we discuss in our manuscript, most prior studies have focused on individual traits or binary trade-offs between a limited suite of traits (e.g., see L90-101). The statement that the reviewer references is one of the key take-home messages from our manuscript—we identified a group of microbial traits that appear to be synergistic for SOM formation. Our results suggest that microbes with intermediate to high trait values across this particular grouping of traits contributed the most to SOM quantity, stability and functional complexity. We feel that this sentence accurately summarizes our findings and is an essential summary of our core results and interpretations—appropriate for a discussion section.

Line 307-309 – First this sentence sounds circular to me, the authors present two explanations for the differences between fungal species in terms of SOM formation: the first is the variation in fungal traits (l308); the second is that differences in SOM formation were linked to variation in fungal traits. Please rephrase and remove repetition. Second, this is a very general sentence for a discussion. In which way did the variation in pathways for fungal-derived SOM affect SOM formation? I suggest being more specific about how fungal species differently contributed to SOM formation based on your own results.

As suggested, we reworded this sentence for clarity (L316-317). It now reads: "In our study, fungal species differed in their contributions to SOM functional pools. Below, we detail how these differences were linked to variation in fungal traits." We hope this helps clarify that this is a transition sentence leading into subsequent paragraphs where we discuss how fungal species contributed differentially to SOM formation in this study.

L310-311 – why is this not hypothesized previously? Perhaps the choice of the different fungal species had this in mind? As I commented before, it is necessary to state why the choice of different species, grouping according to Phyla in the results section, and whether the authors had the generalists vs specialist framework already in mind.

This was not an *a priori* hypothesis; therefore, we did not include it in the Introduction. For this reason, we describe these results as a pattern that we observed among our study species upon analyzing the trait data.

As requested, we added clarification to the Introduction at L130-133 describing the rationale for including the eight fungal isolates that were used in this study. The eight isolates had been studied previously in an experiment where their genomes were sequenced and some of their trait values (e.g., CUE, growth rate) were measured in liquid culture (Morrison et al., 2022, ref. #32). Our goal was to include fungal species that we thought (based on previous work) would span a gradient of trait values, such that we could evaluate continuous relationships between fungal traits and contributions to different SOM pools. This is also described in more detail in the Methods section, but given the structure of *Nature Communications* articles, we appreciate the suggestion to ensure that this is clearly communicated earlier in the manuscript.

L313 – would this also apply to species with low investment in trait categories such as Panellus stipticus, is this a generalist too? Which of the species are specialists and generalists according to the framework used by the authors, and how was this assigned? What is the rationale behind picking intermediate level multifunctionality as a generalist trait and not low ? This seems quite arbitrary from the text, it is important that the author clarify this.

For clarity, we added examples of species that corresponded with the specialist vs. generalist categories at L321 and L323. As we explain at L325-329, functional generalists may have contributed more to SOM formation because species that invest at intermediate levels across a wider range of trait categories (generalists) are more likely to have the genetic and/or physiological capacity for traits important to SOM formation, even if they do not exhibit maximal performance of any given trait. We revised this section of the text for clarity. We hope this helps clarify that we are providing a potential explanation for a pattern that we observed in our results.

Importantly, we are not “picking” (choosing) intermediate level functionality as a generalist trait. We are describing the pattern that we observed across the fungal species included in our study. Specifically, we found that taxa with intermediate (to high) values across a key grouping of traits made the largest contributions to SOM formation and stabilization (across SOM functional pools), and thus, we suggest that these key traits are synergistic for SOM accrual.

L317 – 318 – this is speculation, the data does not test for whether differences in multifunctionality are linked to genetic traits, and importantly, the authors do not build up this argument with other studies. So this statement needs support from relevant literature.

We revised this section of the text for clarity (L325-329) and added a reference to Morrison et al. (2022) (ref. #32) at demonstrating fundamental trade-offs in genetic and/or physiological investment in different traits for the same species we used in our study. This provides a rationale for our suggestion that functional generalists may be more likely to possess the genetic and/or physiological capacity for different traits that are important for the formation of different SOM pools, if they invest at intermediate levels across multiple trait categories. In contrast, functional specialists may be less likely to possess the capacity to perform key traits involved in SOM

formation if they invest at higher levels in a more limited suite of traits. We hope that the edits we made to this section have clarified that this is a suggested interpretation of our results, and a possible explanation for the pattern we observed.

L319-320 – I might have missed it, so could the authors use their own data to support that different fungal traits were associated with the formation of each SOM functional pool? Again, this is extremely general and does not seem to point towards any specific result in the study.

Yes, this is a central result of our paper, which is supported most clearly by the PLSR results presented in Figure 4. Throughout the Results and Discussion, we report and describe how specific traits were associated with the formation of different SOM pools, and collectively, how species that had intermediate to high investment across a key grouping of traits made the largest contributions to multiple SOM pools (i.e., high SOM formation potentials). We hope that the revisions we made to this section (detailed above) help to clarify these points.

L322 – The authors write that "bundles of functions with synergies" expresses a novel ecological concept by Fiedler et al. (2021). But in the paper by Fiedler et al. (2021), this term is used only once throughout the text. The word "bundles" is used two more times as "bundles of services" and "different bundles could be integrated across the landscape". I do not understand what is the new ecological concept "bundles of functions with synergies" the authors refer to when Fiedler at al. (2002) do not refer to it as an ecological concept themselves. "Bundles" means a collection of things, and that is how the words as been used 3 times in that paper. So, this is not a new ecological concept in the paper by Fiedler at all (2021) and this term is introduction confusion and lack of clarity in the discussion. If the authors want to write "bundles of functions with synergies" I suggest the authors with their own words. Please remove this expression in brackets to improve readability in the manuscript.

As suggested, we removed the phrase "bundles of functions with synergies" and rephrased these ideas in our own words (L330-332).

L326 – Please explain what is meant by a "community level syndrome". A syndrome is a collection of symptoms, a combination of things. I don't understand what is meant here or the reply by the authors. In the paper by Malik et al 2019 (reference 28) the word "syndrome" is never used. This is, again, unnecessary jargon that decreases clarity in the manuscript.

We revised this section of the text such that the reference to "community-level syndromes" was removed. We moved this discussion later in the text to the concluding paragraph (L426-434) of the Discussion and now refer to "life history strategies," which is the specific terminology used in the Malik et al. (2019) paper.

L327 – 330 – the authors here criticize the use of binary views such as "high vs low" overemphasizes extreme ends. What do the authors propose instead in this paper? A view with three levels, "low", "intermediate" and "high". How, and using own data can the authors argue that this is a better framework?

In this same section (now moved to concluding paragraph of the Discussion, L426-434), we revised the text for clarity, removing these references to a binary view of "high vs. low" trait values. Based on this reviewer's comment, we realized that this emphasis could cause confusion. We had intended to invoke the idea of a view that emphasizes individual microbial traits (e.g., CUE) or binary trade-offs between trait categories. We are proposing that past frameworks have overemphasized categorical binning approaches that define microbial communities as having a single predominant ecological strategy, thereby limiting our ability to tease out important interactions among microbial traits that fall within different categories (or that are traditionally associated with different life history strategies). Often, these strategies are invoked as if they were mutually exclusive, obscuring the continuous nature of microbial trait expression. We revised this section for clarity, focusing on our recommendations for a more comprehensive assessment of multiple microbial traits and their relationships to the formation of different SOM pools. Such an approach facilitates an understanding of potential interactions and synergies among microbial traits that may be important for the formation of different SOM functional pools.

L337 – 340 – Please provide an explanation for this sentence. Why is SOM amplified at intermediate CUE?

To clarify this point, we added "intermediate to high" CUE to this sentence (L345), such that it is clear we are describing taxa that had intermediate to high CUE values alongside intermediate (to high) values for the other key traits that we identified as being synergistic for SOM formation and stabilization. We hope that this revision, alongside our related explanations above, help to clarify this point.

L337-348 – So is it intermediate (l339-340) or high (l347) trait multifunctionality that lead to higher SOM formation? It seems like it is both but in the end of this set of sentences the author seem to conclude that it is the high trait multifunctionality that leads to SOM formation.

It is high trait multifunctionality that is related to higher SOM formation. This comment seems to stem from a conflation between high "trait values" and high "trait multifunctionality." As we describe at L167-172 (Results) and L318-323 (Discussion), the fungal species with the highest trait multifunctionality scores had intermediate values across many of the measured trait categories.

L 354 – "where C inputs are relatively continuous", please add one or two examples

As requested, we added an example (rhizosphere soil) and added a new relevant citation to the end of the sentence (Sokol et al., 2024; L362-364).

L 359 – 362 – so greater SOM formation potential (l359) was associated with higher necromass decomposition (L360) due to increased mineral-associated SOM? How can the authors connect their own data with mineral reactivity? Also, can the authors explain what is meant by diffusion of residues to soil minerals? The sentence needs rephrasing for clarity.

This sentence states: “We observed greater SOM formation potential among species with a higher capacity for the production of hydrolytic enzymes involved in necromass decomposition (e.g., PHOS, BG, CBH), consistent with the idea that microbial processing of particulate OM can increase the reactivity and diffusion of these fragmented residues to soil minerals[8].” It follows a sentence that describes how more extensive processing of necromass into lower molecular weight compounds may produce residues that are more readily incorporated into stable SOM fractions (refs. 18, 65). These two sentences provide a potential explanation for the relationship that we observed between higher activity of fungal enzymes involved in necromass decomposition and SOM formation potential (which encompassed contributions to relatively stable pools of SOM, including MAOM, chemically and biologically stable C).

L 365 – why would that not lead to stable C forms? Is it because different microbial residues are formed, or what is the explanation? Could this be just species specific, in other words, related to the biomass chemical composition and not to functional traits? Is it CUE that is the core of the discussion or is it turnover rate or chemical composition instead?

This sentence states, “In contrast, if fungi are efficient (i.e., high CUE) but their growth rate is low, they may promote C retention in soils by limiting the amount of soil C lost via respiration, but this C will not necessarily be converted into stable forms.” While it is possible (and likely) that some of these microbial residues will be incorporated into stable SOM pools, the point we aim to make is that CUE does not *necessarily* correlate/correspond directly with microbial contributions to stable SOM pools. CUE is a better indicator of total microbial residue production, with the *potential* for incorporation into different SOM pools (as stated at L348-351). However, other microbial traits (e.g., biomass protein and phenol contents) are likely to influence whether these residues get incorporated into relatively stable SOM pools. This is the basis of the microbial “feedstock trait” and “MAOM formation trait” framework (Sokol et al., 2022, ref. #18), which we provide empirical evidence for in this manuscript.

The focus here is on describing synergies among microbial traits (not on a particular trait like CUE, turnover rate, or chemical composition—but rather their interactive effects).

L377-381 – Does this agree with existing literature or is it a unique finding of the study? Please add that to the text.

This section refers to the “feedstock trait” and “MAOM formation trait” framework by Sokol et al. (2022). As we describe in the Introduction (L120-121), no empirical tests of this framework have been conducted to date. To improve clarity, we revised this section to ensure it is clear that we are (1) referring to hypotheses laid out in the trait framework by Sokol et al. (2022), and (2) that we are describing unique findings of our study, which provide empirical support for the existence of feedstock and MAOM formation traits. The particular section that the reviewer commented on focuses on MAOM formation traits; in L390-402 we go on to situate these results within the existing body of literature that has shown preferential accumulation of proteins and phenols in MAOM and other relatively stable SOM fractions.

L424-427 – this paper does not include plant compounds and their contribution to SOM formation, so it is difficult to understand why the authors try to integrate their results with the

contribution of plant material. That has not been tested and does not link well to the previous sentences.

Thank you for pointing this out. As suggested, we removed this sentence and kept the concluding paragraph focused on microbial traits and inputs to the SOM pool. Revisions can be found at L423-436.

L429-431 The field and readers would benefit from more specific recommendations for future research.

In response to this comment and the preceding comment, we revised the ending of the Discussion (concluding paragraph; L423-436). The recommendations that we make at the end of the paragraph are now more specific and highlight the key take-aways from our study that can inform future research.

Reviewer #3 (Remarks to the Author):

The manuscript has been generally well improved during the revision. Additional justification regarding the usage of glucose as a model compound, further clarified usage of the CUE, an improved terminology, and a strengthened discussion on the microbial production of phenols, among other aspects, have been advanced in the revised version. *Since the manuscript aims to conclude on the formation of soil organic matter, it should be highlighted again that the recovery of MAOM-C and POM-C as proportion of their bulk contents are required (l. 640, Fig. 3b and c, Fig. S4e and f) to assess the representability of these functional pools and their discussion based on correlations with microbial properties.*

We thank this reviewer for their positive feedback on our revisions. After seeing their final comment (italicized above), we realized that we may have misinterpreted a similar comment in their initial review. This earlier comment requested a figure that showed a clear pattern of MAOM and POM "as a proportion of their bulk contents", and in response we created a figure that represented the following: MAOM/(MAOM+POM) or POM/(MAOM+POM), where MAOM + POM proportions summed to 100%. Now that we see this second related comment, we realized that this reviewer was likely asking to see the following: MAOM/total C or POM/total C. We updated Fig. S4 to show MAOM-C and POM-C as a proportion of their bulk (total) soil C concentrations, and we added a new supplemental figure that shows the proportion of total soil C lost during the fractionation procedure (Fig. S5). Thus, for each species, we present a stacked bar plot that sums to 100% = MAOM-C + POM-C + C lost during fractionation (Fig. S5). Carbon loss during fractionation may have occurred due to desorption of MAOM, release of organic matter formerly occluded within the POM fraction or DOC mobilization during the 18h shaking period with sodium hexametaphosphate (standard dispersion protocol prior to wet sieving; see Methods in main text). After dispersion, soils were sieved to separate the < 53 μm fraction (MAOM) from the > 53 μm fraction (POM). Soil suspensions/solutions were then centrifuged, and the supernatant was carefully decanted to isolate the MAOM and POM fractions. Carbon that was not captured within the MAOM and POM fractions was removed with the supernatant.

Our main text figures focus on the amount of C that each fungal species contributed to each SOM pool. These data represent species' contributions to SOM formation and stabilization, and are the focus of our trait-SOM analyses. We agree with this reviewer that it is also useful and informative to present the proportion of total C that was recovered within POM and MAOM fractions. As mentioned above, we include these figures in the supplemental text (Figs. S4 and S5), and we revised/added sentences to the Result section of the main text reporting these results and referencing these figures (L203-206).

Reviewer #4 (Remarks to the Author):

I co-reviewed this manuscript with one of the reviewers who provided the listed reports. This is part of the Nature Communications initiative to facilitate training in peer review and to provide appropriate recognition for Early Career Researchers who co-review manuscripts.

We thank Reviewer 4 again for co-reviewing our manuscript.