Web links to the author's journal account have been redacted from the decision letters as indicated to maintain confidentiality.

1st Jul 20

Dear Dr Buscardo,

Please allow me to apologise for the delay in sending a decision on your manuscript titled "Soil drought effects on soil fungi and biogeochemistry in an Amazon rainforest". It has now been seen by 2 reviewers, whose comments are appended below. You will see that they find your work of some potential interest. However, they have raised quite substantial concerns that must be addressed. In light of these comments, we cannot accept the manuscript for publication, but would be interested in considering a revised version that fully addresses these serious concerns.

We hope you will find the reviewers' comments useful as you decide how to proceed. Should additional work allow you to
• address these criticisms(that is, either to incorporate the suggestions or provide a compelling argument why the point made by the reviewer is not valid, or relevant to the editorial threshold as outlined below)
AND
• meet our editorial thresholds as outlined below,

then we would be happy to look at a substantially revised manuscript. However, please bear in mind that we will be reluctant to approach the reviewers again in the absence of substantial revisions.

In the following, we list our main editorial concerns:

****Provide a clear and compelling argument that extended drought conditions alter the soil microbial community from a P-limited to an N-limited system in tropical forest soils****

****Clearly explain and fully justify all aspects of your methodology, your choice and comparison of experimental treatments, and discuss all limitations****

****Ensure your results are placed clearly in the context of all relevant previous work and include all data required to make informative comparisons with other related studies****

In addition, please supply point-by-point responses to all the reviewers' comments.

If the revision process takes significantly longer than three months, we will be happy to reconsider your paper at a later date, as long as nothing similar has been accepted for publication at Communications Earth & Environment or published elsewhere in the meantime.

We understand that due to the current global situation, the time required for revision may be longer than usual. We would appreciate it if you could keep us informed about an estimated timescale for resubmission, to facilitate our planning. Of course, if you are unable to estimate, we are happy to accommodate necessary extensions nevertheless.

We are committed to providing a fair and constructive peer-review process. Please do not hesitate to contact us if you wish to discuss the revision in more detail.

Please use the following link to submit your revised manuscript, point-by-point response to the referees' comments (which should be in a separate document to any cover letter) and any completed checklist:

[link redacted]

** This url links to your confidential home page and associated information about manuscripts you may have submitted or be reviewing for us. If you wish to forward this email to co-authors, please delete the link to your homepage first **

Please do not hesitate to contact me if you have any questions or would like to discuss the required revisions further. Thank you for the opportunity to review your work.

Best regards,

Joe Aslin

Associate Editor,
Communications Earth & Environment
https://www.nature.com/commsenv/
Twitter: @CommsEarth

EDITORIAL POLICIES AND FORMAT

If you decide to resubmit your paper, please ensure that your manuscript complies with our editorial policies and complete and upload the checklist below as a Related Manuscript file type with the revised article:

Editorial Policy Policy requirements

For your information, you can find some guidance regarding format requirements summarized on the following checklist:
Communications Earth & Environment formatting checklist

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

This manuscript examined the effects of 14 years of consistent rainfall exclusion on tropical forest soil fungal communities and enzyme activities. Rainfall exclusion increased alpha diversity and shifted the composition of fungal communities, increasing the relative abundance of drought tolerant dark septate endophytic fungi. Enzyme activities also shifted from P to N acquisition under seasonal drought conditions.

This is an impressive amount of work and one of the only studies to examine long-term belowground responses to drought in tropical ecosystems. Nevertheless, I have several comments to improve the impact of this work.

Hypotheses: These seem to come from nowhere. I suggest introducing fungal drought response earlier in the introduction to set up the reader for H1 and H2. It's also unclear why H1 and H2 are presented separately. Maybe use H2 as H1a or say something like "an alternative hypothesis". In particular dark septate endophytes should be presented earlier. Similarly, H3 is about enzyme activities but this is the first time they are mentioned. Some background would allow the reader to place this prediction in the proper context. I also suggest that the introduction focus on fungi if that is what is being measured instead of microbial communities writ large.

Introduction: I would add a sentence or two about the experimental design and sampling scheme since the structure of this paper jumps from the introduction to the results. Specifically details about sampling in the wet v. dry season and the number of plots would be helpful.

Methods: Overall it's unclear how treatments were compared for all analyses that did not include composition. How do the t-tests mentioned on Page 10 Line 3 square with the ANOVAs listed at the end of this section? Why not run a full model with both treatment and time/season using repeated measures ANOVA? If t-tests are the only way, were they corrected for multiple comparisons? I only see mention of this in the ANOVA section.

Page 9, Line 35: Why were the data not rarefied? If not, where they transformed to proportions to account for unequal sampling depth?

Page 9, Line 37: It seems like only half of the fungal taxa were able to be assigned to functional groups in FUNGuild. This is fine, but you need to point this out in the discussion and how this could bias any functional results (e.g., what if all of the unassigned were actually decomposers and then the relative abundance of DSE was much lower).

Page 10, Line1: There is a typo here.

Results/Discussion: It's somewhat challenging to match the results to described methods and figures. For instance, I don't think nestedness is described at all in the results. Statements like Page 4 Line 3 could be refined to state which treatments differed. Was diversity highest in the drought treatments in the dry season? Which treatments/seasons varied in enzyme activities? It is very difficult to compare the figures to the text here and elsewhere. The text seems to assume that we know what the figures and figure legends say already.

Drought effects: It seems like the highest richness occurred in the control treatments in the dry season, not in the drought treatments in the dry season. Actually, it looks like the drought treatments had pretty consistent and median diversity regardless of season. How does this match

H1? Shouldn't that require that the highest diversity occurs in the dry plots in the dry season? Actual rainfall data here may help as instantaneous soil moisture may not match what the fungal community responded to (i.e. compositional/richness shifts may take time and respond to moisture pulses from the week or month before.). Similarly, enzyme results are largely derived from shifts in the control plots between seasons. How does this reconcile with H3 where the drought treatment should have the largest effect?

Page 5, Line 15: Here and elsewhere the treatments are referred to as "extreme drought". This should be rephrased to drought treatments in the dry season or something similar to match the experimental design in the methods.

Figures:
Figure 1: I really like the idea of this figure, but it seems like you could use empirical data to really show that your treatments and rainfall seasonality imposed this type of soil moisture response. What was the rainfall like in the year that you sampled? Was it typical of the MAP for this region as you report in the text or drier that normal, etc.?

Figure 2: Were separate stats run for each functional group? If not, they should be.

Figures 3,4, and 5: It's unclear why you needed paired t-tests here instead of comparing all treatments/seasons simultaneously with an ANOVA like the end of the methods section suggests.

Figure 4: You say that you have ~2600 OTUs and that half of them were defined to functional group. Why is the species richness here so low then? Even if composition was completely different among treatments, I don't think these numbers would add up to ~1300.

Reviewer #2 (Remarks to the Author):

The effects of drought and climate change on tropical forests is an important topic for forest management and global carbon dynamics. The paper includes interesting and novel observations. Yet the paper itself is missing data, context and comparisons that make it difficult to assess.

No taxonomic breakdown of fungal community composition is given, even though the authors have the data. That information has high interest for those studying fungal biogeography.
Why are no mycorrhizal groups included in the functional group breakdown?
A large chunk of the text is devoted to the observation that dark septate fungi (DSF) increase with drought, reaching 7.5% of "identified functional groups" (not total OTUs? Not clear what this means). Some of that text is a digression on fungal dispersal and evolution that adds nothing to the paper.
The significance of the DSF result is not clear. DSF abundance generally increases with aridity, so is the DSF response surprising? The authors write that DSF have not been reported previously in these tropical forests, but do not state whether anyone has looked. A broader context is needed. What were the dominant DSF taxa? How does DSF community composition compare to those from other areas? Does the low soil pH reduce DSF diversity?

Perhaps unlike the fungal analyses, there are abundant data on EEA (and their ratios) in tropical systems. But no comparisons with other studies are included to place these results in context. The EEA data are presented in Figure 5 without units. The methods and supplemental info do not include units either. Interpretation of EEA results differs depending on whether activities are normalized to dry mass, organic matter or microbial biomass. The data collected appear to allow any or all of these to be calculated. Again, the lack of context and units limits the value of the study for those looking for larger scale trends.

The authors provide data on several edaphic factors that might influence the results, but no information on NPP responses is provided except for fine root stock. Changes in NPP and canopy composition might underlie the observed responses.

Dear Reviewers,

On behalf of all authors, I would like to thank you for your constructive criticism and insightful comments that helped us revise and improve our manuscript. We believe that we have now satisfactorily addressed all points raised.

We have inserted the replies in blue typeface after each comment. The changes in the manuscript text are marked in yellow.

Thank you again.

Yours sincerely,

Submitting author

REVIEWER COMMENTS:

Reviewer #1 (Remarks to the Author):

This manuscript examined the effects of 14 years of consistent rainfall exclusion on tropical forest soil fungal communities and enzyme activities. Rainfall exclusion increased alpha diversity and shifted the composition of fungal communities, increasing the relative abundance of drought tolerant dark septate endophytic fungi. Enzyme activities also shifted from P to N acquisition under seasonal drought conditions.

This is an impressive amount of work and one of the only studies to examine long-term belowground responses to drought in tropical ecosystems. Nevertheless, I have several comments to improve the impact of this work.
We thank the reviewer for his/her appreciating our work and for the insightful comments, to which we reply in detail below.
Hypotheses: These seem to come from nowhere. I suggest introducing fungal drought response earlier in the introduction to set up the reader for H1 and H2. It's also unclear why H1 and H2 are presented separately. Maybe use H2 as H1a or say something like "an alternative hypothesis". In particular dark septate endophytes should be presented earlier. Similarly, H3 is about enzyme activities but this is the first time they are mentioned. Some background would allow the reader to place this prediction in the proper context. I also suggest that the introduction focus on fungi if that is what is being measured instead of microbial communities writ large.
We have extensively reworked the Introduction, following the reviewer's comments and the Instructions for Authors of this new journal. Please see Page 2, Lines 23-42.
We have reframed our hypotheses, the new H_1 combines the old H_1 and H_2, and the new H_2 refers to enzyme activities. Please see Page 3, Lines 29-41.
Introduction: I would add a sentence or two about the experimental design and sampling scheme since the structure of this paper jumps from the introduction to the

results. Specifically details about sampling in the wet v. dry season and the number of plots would be helpful.

Done – see Page 3, Lines 41-44.

Methods: Overall it's unclear how treatments were compared for all analyses that did not include composition. How do the t-tests mentioned on Page 10 Line 3 square with the ANOVAs listed at the end of this section? Why not run a full model with both treatment and time/season using repeated measures ANOVA? If t-tests are the only way, were they corrected for multiple comparisons? I only see mention of this in the ANOVA section.

Thank you for pointing out this discrepancy in data analysis. We now present all results as t-tests or Wilcoxon signed-rank tests, with corrected multiple comparisons – the statistical significance was not affected in any of the cases. The authors had spent considerable time before submission, discussing the experimental set-up and the statistical treatment of the data. In a first approach, the use of a nested design would indeed appear to be an elegant approach. However, one could argue that as in any study that uses destructive sampling (in our case, removal soil of core), sampling the exact same point in space is not possible (unlike the case with litterfall traps, for example) – although to counter this we sampled within close vicinity of the original. Furthermore, given the fact that our data series (seasons) consist of two sets of measurements only, we decided that comparisons using t-tests with adjusting for multiple comparisons would be the safest and most transparent (easy-to-trace) way to present our results. Paired t-tests or Wilcoxon signed-rank tests were corrected for multiple comparisons using the false discovery rate method (see Materials and Methods Page 14, Lines 3-6 and relevant figure legends).

Page 9, Line 35: Why were the data not rarefied? If not, where they transformed to proportions to account for unequal sampling depth?

The NMDS ordinations of the fungal community and on the main functional groups were made on both non-rarefied presence/absence (P/A) and relative abundance (RA) data (see Fig 2 for NMDSs performed on P/A data and Supplementary Fig 2 for NMDSs performed on RA data), and rarefied P/A and RA data (see Supplementary Fig 1 for NMDSs performed on P/A and RA data). PERMANOVA was also conducted on both non-rarefied and rarefied P/A and RA data (see results in Page 4, Lines 36-39 and Page 5, lines 19-21 for results obtained from non-rarefied P/A data and Supplementary Figs 1-2 for those obtained on non-rarefied RA and rarefied P/A and RA). Since the results of these analyses did not present appreciable differences, seasonal and treatment differences in richness and relative abundance of fungal communities and functional groups were made exclusively on non-rarefied data.

Page 9, Line 37: It seems like only half of the fungal taxa were able to be assigned to functional groups in FUNGuild. This is fine, but you need to point this out in the discussion and how this could bias any functional results (e.g., what if all of the unassigned were actually decomposers and then the relative abundance of DSE was much lower).

We have now included this information including a cautioning statement regarding the proportion of functional groups – see Materials and Methods Page 13, Lines 15-18.

Page 10, Line1: There is a typo here.

Thank you – corrected now

Results/Discussion: It's somewhat challenging to match the results to described methods and figures. For instance, I don't think nestedness is described at all in the results. Statements like Page 4 Line 3 could be refined to state which treatments differed. Was diversity highest in the drought treatments in the dry season? Which treatments/seasons varied in enzyme activities? It is very difficult to compare the figures to the text here and elsewhere. The text seems to assume that we know what the figures and figure legends say already.

We have completely reworked the text by adding separate Results (Pages 4-6) and Discussion sections as a result of the reviewer's comments and following the Instructions for Authors for this journal. We believe that it has much improved the readability of the text.

Drought effects: It seems like the highest richness occurred in the control treatments in the dry season, not in the drought treatments in the dry season. Actually, it looks like the drought treatments had pretty consistent and median diversity regardless of season. How does this match H1? Shouldn't that require that the highest diversity occurs in the dry plots in the dry season? Actual rainfall data here may help as instantaneous soil moisture may not match what the fungal community responded to (i.e. compositional/richness shifts may take time and respond to moisture pulses from the week or month before.). Similarly, enzyme results are largely derived from shifts in the control plots between seasons. How does this reconcile with H3 where the drought treatment should have the largest effect?

We have clarified this in the text (see added Results section) in the light of our revised hypotheses (intermediate disturbance *vs.* long-term press (high) disturbance).

We have included the rainfall data for the year of the study and means with SD for the entire period of the experiment (Fig. 1) to underpin our hypotheses and present an overall picture of rainfall seasonality.

Page 5, Line 15: Here and elsewhere the treatments are referred to as "extreme drought". This should be rephrased to drought treatments in the dry season or something similar to match the experimental design in the methods.

We have defined and standardised the reference to non-extreme drought and extreme drought. See Introduction Page 4 Lines 1-5 and legend Fig. 1.

Figures:

Figure 1: I really like the idea of this figure, but it seems like you could use empirical data to really show that your treatments and rainfall seasonality imposed this type of soil moisture response. What was the rainfall like in the year that you sampled? Was it typical of the MAP for this region as you report in the text or drier that normal, etc.?

Thank you for the suggestion - Done

Figure 2: Were separate stats run for each functional group? If not, they should be.

Thank you for this observation. Initial statistical analyses were related only to the total fungal community as described in the Figure legend of the original version of the manuscript. We have made additional analyses for each functional group. See results Page 4, Lines 39-41 and Page 5, Lines 21-25.

Figures 3,4, and 5: It's unclear why you needed paired t-tests here instead of comparing all treatments/seasons simultaneously with an ANOVA like the end of the methods section suggests.

See our reply above.
Figure 4: You say that you have ~2600 OTUs and that half of them were defined to functional group. Why is the species richness here so low then? Even if composition was completely different among treatments, I don't think these numbers would add up to ~1300.
Total richness for each functional group per season/treatment is represented by the numbers in the box plots. These numbers (632 for C wet; 617 for T wet; 697 for C dry; 654 for T dry) if added up, *i.e.* if there were a complete turnover across treatments/seasons , would give *ca*. 2,600 OTUs.
Beta diversity (0.61 – 0.71) had a turnover component that ranged between 85 and 94%. Moreover, we clarify in the text that 256 of the 1,335 OTUs that were assigned to functional groups belonged to multiple functional groups and were therefore not considered in this figure. This information was added to the text Page 13, Lines 15-18.

Reviewer #2 (Remarks to the Author):

The effects of drought and climate change on tropical forests is an important topic for forest management and global carbon dynamics. The paper includes interesting and novel observations. Yet the paper itself is missing data, context and comparisons that make it difficult to assess.
Thank you for your appreciating our study and for pointing out improvements required.
No taxonomic breakdown of fungal community composition is given, even though the authors have the data. That information has high interest for those studying fungal biogeography.
We now provide phylum level information – see Page 4, Lines 21-29; Page 5, Lines 6-18. See also Supplementary Fig. 3.
(More in-depth taxonomic treatment was beyond the objective of this study and a detailed taxonomic treatment will be provided soon in a taxonomy focused paper.)
Why are no mycorrhizal groups included in the functional group breakdown?
Symbiotrophs, including mycorrhizal fungi, endophytes and lichenised fungi were represented by a total of 42 OTUs, i.e. 3.1% of the OTUs classified by FUNGuild and they did not change with seasonality or treatment. For this reason, they were not included in Fig 4. This information however is now added to the Results section (Page 5, Lines 31-33).
A large chunk of the text is devoted to the observation that dark septate fungi (DSF) increase with drought, reaching 7.5% of "identified functional groups" (not total OTUs? Not clear what this means). Some of that text is a digression on fungal dispersal and evolution that adds nothing to the paper.
We have extensively revised and much reduced the text on dispersal / evolution to not to digress.
DSF constituted the 7.5% of OTUs assigned to individual or multiple functional groups (see also reply to reviewer 1). We did not calculate the percentage of DSF (or of the other functional groups) on total OTU richness, *i.e.* OTUs assigned and unassigned to a

group by FUNGuild, because this figure would not clearly or accurately reflect the proportions of each functional group.

The significance of the DSF result is not clear. DSF abundance generally increases with aridity, so is the DSF response surprising? The authors write that DSF have not been reported previously in these tropical forests, but do not state whether anyone has looked. A broader context is needed. What were the dominant DSF taxa? How does DSF community composition compare to those from other areas? Does the low soil pH reduce DSF diversity?
DSF have been reported to extensively colonise plants in stressful and nutrient-limited environments (*e.g.* polar and alpine habitats); with respect to temperature and water availability, this is not usually the case with humid tropical rain forests. We found only a single study that deals with DSF in a tropical rain forest: in particular it considered the diversity of DSF in tree roots in the Atlantic Forest[1]. However, we couldn't find any data on the relative abundance of this functional group in relation to other soil fungi/functional groups. Moreover, available studies on fungal communities in tropical rain forest that we are aware of, do not make reference to DSF at all. This group could possibly be overlooked due to the fact that the interpretation of results based on taxonomic classification often fails to take into consideration functional/ecological aspects. It is also true that the knowledge on microbial diversity (both taxonomic and functional) in tropical rain forest soils is still scarce compared with what is known in other ecosystems, mainly in the Northern Hemisphere.
The closest match of OTUs belonging to the DSF group was *Oidiodendron*. However, the sequence matches ranged from 70 to 99% with almost 60% of the OTUs having a similarity sequence match below 95%.
Perhaps unlike the fungal analyses, there are abundant data on EEA (and their ratios) in tropical systems. But no comparisons with other studies are included to place these results in context. The EEA data are presented in Figure 5 without units. The methods and supplemental info do not include units either. Interpretation of EEA results differs depending on whether activities are normalized to dry mass, organic matter or microbial biomass. The data collected appear to allow any or all of these to be calculated. Again, the lack of context and units limits the value of the study for those looking for larger scale trends.
Thank you for pointing these issues out. We have carefully combed the literature for all EEA information in neotropical forest ecosystems (page 9, lines 23-37). In the Discussion, we have made comparisons across studies based on EEA values standardised for SOM. We apologise for the lack of units in Fig 5. – it was an embarrassing oversight on our part.
The authors provide data on several edaphic factors that might influence the results, but no information on NPP responses is provided except for fine root stock. Changes in NPP and canopy composition might underlie the observed responses.
The near continuous exclusion of about 50% of the rainfall over a period of 14-years represents an ecosystem that after initial serious structural and functional changes has now stabilised and is no different in net primary productivity (NPP) of trees > 10 cm dbh from its paired control[2], with more young smaller trees making up for the loss of some

large trees in the droughted plot. At this point in time it is valid to ask what changes, if any, in the biotic community might have contributed to this stabilisation of NPP, through compensatory mechanisms for nutrient and water acquisition in the droughted plot, very probably mediated by positive biotic interactions not accounted for in studies on the population of trees > 10 cm dbh. Subsequent work has shown evidence of stabilisation in litterfall dynamics and was less clear on biomass dynamics. Full test with growth and litterfall is forthcoming.

REFERENCES cited in replies

1 Bonfim, J. A., Vasconcellos, R. L. F., Baldesin, L. F., Sieber, T. N. & Cardoso, E. J. B. N. Dark septate endophytic fungi of native plants along an altitudinal gradient in the Brazilian Atlantic forest. *Fungal Ecology* **20**, 202-210, doi.org/10.1016/j.funeco.2016.01.008 (2016).

2 da Costa, A. C. L. *et al.* Ecosystem respiration and net primary productivity after 8–10 years of experimental through-fall reduction in an eastern Amazon forest. *Plant Ecology & Diversity* **7**, 7-24, doi:10.1080/17550874.2013.798366 (2014).

17th Dec 20

Dear Dr Buscardo,

Pleas allow me to apologise for the delay in sending a decision on your manuscript titled "Natural and experimental soil drought effects on soil fungi and biogeochemistry in an Amazon rainforest". It has now been seen by our reviewers, whose comments appear below. In light of their advice I am delighted to say that we are happy, in principle, to publish a suitably revised version in Communications Earth & Environment under the open access CC BY license (Creative Commons Attribution v4.0 International License).

We therefore invite you to edit your manuscript to comply with our format requirements and to maximise the accessibility and therefore the impact of your work.

EDITORIAL REQUESTS:

Please review our specific editorial comments and requests regarding your manuscript in the attached "Editorial Requests Table". Please outline your response to each request in the right hand column. Please upload the completed table with your manuscript files.

If you have any questions or concerns about any of our requests, please do not hesitate to contact me.

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We hope to hear from you within two weeks; please let us know if you need more time.

Best regards,

Joe Aslin

Associate Editor,
Communications Earth & Environment
https://www.nature.com/commsenv/
Twitter: @CommsEarth

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have addressed all of my previous comments.

Reviewer #2 (Remarks to the Author):

The revised paper is much improved. The organization of the introduction is better. The statement and rationale for the hypotheses are clear. Including an overview of the experimental design helps readers avoid flipping back and forth to the methods section. (I have never understood why journals adopt that frustrating organization style). The results have been completely rewritten in an easy to follow and logical manner. The discussion includes more theoretical and comparative context for the results. The figures are well done and helpful. The paper is a significant contribution to the literature, likely to be widely cited and ready for publication.

REVIEWERS' COMMENTS:

Reviewer #1 (Remarks to the Author):

The authors have addressed all of my previous comments.

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We thank again the reviewers for their constructive input that helped improving the content and clarity of presentation of our manuscript.