Biogeosciences Discussions

Biogeosciences Discuss., referee comment RC1
https://doi.org/10.5194/bg-2022-83-RC1, 2022

Comment on bg-2022-83

Mark Anthony (Referee)

Referee comment on "Nitrophobic ectomycorrhizal fungi are associated with enhanced hydrophobicity of soil organic matter in a Norway spruce forest" by Juan Pablo Almeida et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2022-83-RC1, 2022

Overall summary:

This was a lovely paper to review chock-full of interesting fungal ecology. The authors explore the establishment of fungal communities into mesh-bags with sand installed at a Norwegian boreal forest from control and nitrogen addition plots. They explored changes in hydrophobicity of in-growth bags and how this was linked to the ectomycorrhizal fungal community over three years of sampling. They provide evidence that the proliferation of particular ectomycorrhizal fungi, like Piloderma olivaceum, could be what causes hydrophobicity to increase over-time in the mesh bags. Because it is not only a long-distance explorer but also possesses interesting mycelial chemistry, such as mycelium coated with calcium oxalate, there is good theoretical support for this idea. To my knowledge, this is quite a nice and novel finding, with important implications for soil C storage. They also show that N additions prevented increases in hydrophobicity, shifted fungal communities at later stages, and prevented P. olivaceum from establishing. It's always nice to review a paper clearly written by people who are knowledgeable and care about fungal ecology and who had a clear question to ask, which makes it quite unique from the lion's share of ITS-based DNA-metabarcoding studies.

I have no high-level concerns for the paper but a number of important points regarding specific sections below in the line-by-line comments. I hope these improve the paper even further.

Sincerely,

Mark Anthony

Line-by-line comments:

Line 56: change 'to' to 'for'

Line 57: I have been curious of this framing of these results because ericoid mycorrhizal fungi include many very strong decomposers (e.g. Burke and Cairney 2002, Mycorrhiza; Kohler et al. 2015, Nature Genetics)

Line 61: Though a great study, I would not say that Lindahl et al. (2021) could conclude causality in their work, and thus I would not say that C. acutes 'resulted in...'. Rather, I would say 'was linked to'.

Line 65-68: These results by Lilleskov et al. (2011) are very important, but some of the summaries at the genus level need to be reconsidered. For example, increasing evidence suggests that members of the Russula and Lactarius genera include species that respond both negatively and positively to N additions (e.g. Morrison et al. 2016, Fungal Ecology; Van der Linde et al. 2018, Nature; Moore et al. 2021, GCB; Anthony et al. 2021, ELEMENTA).

Line 78-83: Very interesting and nice expectations for the results plus clearly stated. Nice!

Line 94-95: Each fertilisation treatments includes a 50 kg N ha-1 yr-1 range, why is this? Maybe adding one additional clause to clarify. Because you ultimately consider fertilisation a single treatment, it is easily defendable but it would be good to stand lone in the paper versus needing to read Bergh et al. (2008) first. Can you also describe how long the N addition treatment was fertilized prior to installing the mesh bags?

Line 95-96: Can you provide more information on the fertilisation of other macro- and micronutrients? Because micronutrient loss is also hypothesised to influence how fungi respond to N additions (e.g. Whalen et al. 2018, GCB).

Line 116: It would be interesting to know why in November versus a time when tree growth and belowground C allocation is presumably higher.

Line 154: Please add one sentence about how sequences were denoised, given this is 454 data it is especially important bioinformatic detail.

Line 169-172: Was this part of the work done manually?

Lien 174: I realise the bioinformatics was done many years ago, but because the submission is current, I encourage updating language around the Zygomycota to be consistent with current taxonomic consensus (see Spatafora et al. 2016, Mycologia).

Line 175: Does 'unknown ectomycorrhizal status' also refer to non-ectomycorrhizal taxa or just taxa thought to be ecto but not confirmed?

Line 180-182: Maybe just say 'relative abundance'?

Line 184-198: I am not well-qualified to evaluate this method but it seems rather intuitive and appropriate.

Line 215: Can you also provide technical details on the C/N measurements and the ergosterol analysis?

Line 221: Was this a PCA of all these variables together or was some type of vector fitting used to fit the non-fungal values (e.g. envfit function in vegan)? I am also a bit concerned of using PCA on relative abundance data given how many zeros are probably in the data and co-linearity among some of the taxa. Is there a reason why PCoA was not used with Bray-Curtis distance or a distance-based redundancy analysis also using Bray-Curtis dissimilarity? Both of these would be more suitable non-parametric alternatives. I am also guessing from Figure 4 that this is a PCA of both fungal relative abundances and organic matter properties together. Thus, was some type of transformation used to put everything on the same scale? Additionally, can you define what was the criteria for being the most abundant fungal OTU. Was there a cutoff based on sequence proportion and/or occurrence across the sampling units? I am also guessing this is at the OTU level?

Line 257: I think the alphabetic labels are missing on the figure (e.g., a, b, & c).

Line 268: I would also be interesting to have the Pearson correlation coefficient here.

Line 271: I think Figure 2 could be cleaned up a bit so the legend and axis labels look nicer and do not contain underscores.

Line 285: Why is it total fungal EMF and saprotrophic fungal communities? Is this because all other trophic groups and non-assigned to trophic group fungi were removed? Did you also look at EMF alone and saprotrophs alone?

Line 301-304: These details seem like they should be the first part of section 3.4, where the molecular results are first introduced.

Line 305-310: Already stated verbatim in section 3.3?

Line 311-316: Already stated at lines 290-294.

Line 321. Missing period after '(Fig 3b)'

Line 331-333: What linear model was used? Please add these details into the methods section.

Line 333-334: I do not believe you mean to say the 'proportion of EMF to total Basidiomycota', as it sounds like you calculated a ratio of the two, but rather, I think you mean to to say, 'the proportion of EMF and the proportion of EMF increased over-time…'. I would also write Basidiomycota and Ascomycota versus 'mycetes'

Section 3.5: You only need to site Fig. 4 one time.

Line 358: The idea of fungal succession is important, but it need not be independent from changes in environmental conditions across time. I personally do not feel this qualification is necessary here. It derails the momentum of your story so early on! If you feel it is essential to already provide a caveat in this first paragraph of the discussion, I encourage you make one that does not derail the traction of the main conclusion of this work. You could say something more powerful like: 'Whether shifts in EMF were due to selection of later succession fungal taxa as the forest aged versus variation in climatic conditions remains unclear, but is ultimately not particularly important in terms of understanding how shifts in EMF relate to soil organic matter cycling'.

Line 359-363: Very nice and strong result! I find it very interesting.

Line 394: I believe this result is robust around T. asterphora responding positively to N additions, but I am willing to hedge it is geographically unique. Van der Linde et al. (2018. Nature) suggest an N depo optimum around 9 kg N ha yr-1 for this taxon, which is quite low for many parts of Central Europe where the work was conducted. Since your work was more northern and in a boreal forest, I bet the results are quite different from more southern, temperate forests. This is just a comment for consideration. I do not think there is need to comment on this in the text.

Line 401: Could you explain why the N tolerance component of this sentence helps to explain this result in little more detail?

Line 404: You found the hydrophobicity did not change until the third year in the control mesh bags, which is also when proportions of EMF dramatically increased to make this group dominant. This supports the idea that the increase in hydrophobicity was due to EMF accumulation. It could be worth stating this also in this paragraph.

Line 421-441: Such an interesting paragraph! The explanation around yeasts and their ergosterol content is particularly compelling.

Line 442-473: Another very interesting paragraph! Good ideas to explain the role of Piloderma species in soil C cycling.

Figure 1. Is this the average contact angle at 1 and 5 s combined?

Figure 2. It could help to provide a sentence describing what CA at 1 s and 5 s means; otherwise, the figure is a little tricky to interpret as a stand-alone component.

Figure 3b: It is hard to differentiate between Tomentella stuposa and Tylospora asterophora and then between Piloderma olivaceum and Amphinema byssoides and then between Suillus lutes and Amphinema sp 5. Could you select colors with more contrast?

Figure 4: Are the vectors the coefficients of the linear combos of the initial variables used to make the PCA? I would also write OTU instead of species.