Biogeosciences Discussions

Biogeosciences Discuss., referee comment RC3
https://doi.org/10.5194/bg-2022-83-RC3, 2022

Comment on bg-2022-83

Anonymous Referee #3

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-RC3, 2022

Ectomycorrhizal fungi (EMF) have varied potential effects on SOM properties and decomposition. Some EMF produce large quantities of hydrophobic mycelia, which may increase the recalcitrance of SOM, enhancing soil C stocks. Not all EMF produce hydrophobic mycelia, however. N fertilization, for instance, has been shown to select against hydrophobic EMF, potentially reducing the effects of EMF fungal ingrowth on SOM hydrophobicity and recalcitrance. This study performs a number of analyses on sand-filled meshbags amended with maize incubated for 2 years and 8 months over a full fertilization experiment (~50-75 kg N/ha/yr + other macro-/micronutrients) to test the effects of the EMF community on the hydrophobicity of the meshbag contents. While I found the aims of this study compelling, several aspects give me pause. I am particularly interested in how this study parses between the effects of the full fungal community vs EMF on substrate hydrophobicity.

I think more explicit treatment of EMF community composition would strengthen the claim that the hydrophobicity unique to some EMF taxa is responsible for increased substrate hydrophobicity in the control plots. For instance, Almeida et al. could code for mycelial hydrophobicity and compare the relative sequence abundance of hydrophobic EMF taxa over time/across treatments. The effect of fertilization is particularly important given that the design of the study hinges on the idea that EMF with hydrophobic mycelia decline with fertilization. I would also like to see more information about the variability within the EMF community. For instance, instead of a stacked bar chart, Figure 3b could be broken out by treatment and each taxon could have an error bar. Further, much of the discussion centers on the physiology and ecology of the most abundant species of EMF in the control plots (*Piloderma oliviceum),* but little information is available regarding how consistently this taxon shows up in the meshbags. If *P. oliviceum* is indeed abundant across most samples, this would strengthen the discussion of its role in potentially enhancing SOM hydrophobicity. Also, if possible, regressing the relative sequence abundance of hydrophobic EMF against the averaged contact angle of the substrate in an ANCOVA would strengthen the claim that EcMF mycelial hydrophobicity is imparting increased hydrophobicity to meshbag contents. If this emerges across N fertilization treatments, this would be particularly impactful.

45-47: The hydrophobicity of living EMF mycelia is framed as a possible driver of SOM hydrophobicity here, but the subsequent analyses do not address how hydrophobic vs. hydrophilic EMF differ in abundance over the fertilization treatments. Is there a way to either change this framing, or address it with further analyses?

48-50: I'm unclear about how by removing N and P from SOM, EMF activity may reduce soil C stocks. By inhibiting saprotrophic activity by outcompeting them for nutrients, wouldn't this enhance soil C stocks by reducing respiration by saprotrophic fungi (Gadgil effect)? Do you mean that EMF themselves are mineralizing C from SOM, decreasing soil C stocks?

81-83: Here, you write that you would expect higher hydrophobicity in the control vs. fertilized plots due to the higher proportion of hydrophobic species – where is this tested? Genus-level assignments on mycelial hydrophobicity are available in the literature, as well as exploration type assignments that could offer further resolution on the effect of EMF mycelial traits on substrate hydrophobicity.

235: Table 1 would benefit from indicators of significance, either between treatments or over time. I found myself asking questions like "is the decline in the contact angle in the fertilized plots over time significant?" and struggling to locate the relevant information in the text. Alternatively, it could be visualized, which would also make it easier to interpret.

258-260: Looks like you're missing figure labels (a, b, c).

272-273: Figure 2, would it be possible to make this a little cleaner (axis titles, the legend title)? Also, when the proportion of EMF reads is so low during the first two harvests, how can you attribute new C (C3 ingrowth into C4 substrate) to EMF alone? Table 1 indicates that roughly half of the "new" carbon enters the substrate by the end of the first incubation, although the proportion of EMF reads is only ~ 10%.

296: Figure 3, could you break panel b out to provide more information about how variable the EMF community is? Also, could you provide visual information about how the traits of the EMF community (hydrophobicity and/or exploration type) may be shifting according to sequencing results?

300: How do different kinds of EMF respond to the fertilization treatment over time? Hydrophobic vs. hydrophilic genera?

359-363: Here you argue that overall EMF abundance is linked with higher hydrophobicity. This is different from your original framing, where you implicate EMF producing

hydrophobic mycelia.

372-374: What is the mechanism of partner selection implied here? Reduced total C allocation to EMF fungi? How do you rule out environmental filtering and/or changes in EMF C sink strength? The Defrenne study cited here is correlative – how does it support your causal claim?

375-376: This is where I think more information about the homogeneity vs. patchiness of the EMF community would be helpful – are *Piloderma* spp. abundant across all samples?

415-419: Refer to comment from line 235. How can you attribute new C to EMF when their relative abundance after the first incubation was so low? Also, the synthesis offered here (EMF necromass and biomass may contribute to SOM hydrophobicity) deviates from your original hypothesis, that EMF with hydrophobic mycelia in particular are contributing to SOM hydrophobicity.

437-439: This argument suggests that filamentous fungi contribute more than yeasts to SOM hydrophobicity – that is a much larger group than EMF fungi. How can you parse between the effects of filamentous fungi at large and EMF?

445-449: This discussion would be strengthened by a more robust quantitative analysis of the relative abundance of hydrophobic/hydrophilic EMF across treatments. Where is your final hypothesis addressed?