

Christopher Fernandez' comments (Reviewer 2):

Thank you Christopher for your comments. They definitely improved the manuscript! Especially the suggestion of coding the EMF species according to their exploration type and hydrophobicity.

Comment:

How do you tease apart the effects of fungal in-growth from hydrophobic compounds entering the bags from the surrounding litter/SOM (e.g. ingress of hydrophobic compounds via transport in water)? While I think the evidence presented in the manuscript strongly suggests that the former is probably the major driver, I don't think one can completely rule out the potential effects that the surrounding litter and SOM (and associated changes with the N treatment) may have on the substrate properties in the in-growth bags. I would suggest adding a few sentences in the discussion about plausible alternative mechanisms.

Answer:

This is a very good point. I agree that we cannot rule out the possibility that soil solution entered the meshbags during the incubation inside the soil.

However there is some indication that EMF might explain the new C inside the bags since the number of EMF reads was significantly correlated with the new carbon in the meshbags (Pearson, $T = 2.4$, $p < 0.05$). **I have added this result (not mentioned before) in the manuscript.**

I added a couple of sentences in the discussion stating that:

It should be also noted that we cannot rule out the possibility that other compounds from the soil entered the meshbags during the underground incubation. In soils, polymeric substances coming from SOM, root or microbial exudates can have hydrophobic properties (Vogelmann et al., 2013; Mataix et al., 2007). Hence, the hydrophobic changes in the material could be partly explained by other sources than EMF mycelium. However, the significant correlation between the new carbon in the bags and the EMF reads and the negative effect of fertilization on the C.A might suggest that hydrophobicity changes in the meshbag content are caused mainly by EMF.

Comment:

L3 For correct grammar change “fungi” to “fungal” OR just omit “symbiosis”

Answer:

Done.

Comment:

L37 revise to say “...this mycelium turns into necromass...”

Answer:

Done.

Comment:

L44 the authors might want to add hypothesized mechanisms behind differences in decomposition rates among hi and ho SOM here

Answer:

I added a short explanation about it:

SOM can be protected from decomposition in aggregates where hydrophobic coatings of mineral particles change the physical properties of the particles, reduce water films around them and limit water penetration inside the aggregates. This affects the mobility of microbial decomposers and enzymes from the soil solution and reduces organic matter decomposition.

Comment:

L53 change “saprophyte” to “saprotroph” for consistency (and a more widely accepted term)

Answer:

Done.

Comment:

L60-63 I would suggest explicitly stating that this particular species of Cortinarius has retained the enzymatic capacity to breakdown complex SOM in order to access nutrients.

Answer:

I added:

Certain species of EMF may have exceptional importance for organic matter degradation as the presence of Cortinarius acutus (which has retained the enzymatic capability to breakdown SOM to access nutrients) was linked to 33% lower C storage in the organic top soils in 359 investigated stands in boreal forests in Sweden (Lindahl et al., 2021).

Comment:

L64-68 species are mentioned but genera are given as the examples

Answer:

Replaced the word species by EMF.

Comment:

L64-68 I would add a sentence stating that for Russula and Lactarius there is quite a bit of variability in response to N fertilization at the species level

Answer:

We removed these genera

Comment:

L66 missing a “,” in front of Suillus

Answer:

Fixed.

Comment:

L92-96 Please provide what form the N fertilizer was

Answer:

A couple of sentences were added:

In the fertilization treatments specific amounts of N (ammonium and nitrate) were applied to optimize plant growth without inducing leaching. The amount of N additions were based on needle N determinations and monitoring of N in soil water (Bergh et al 2008).

Comment:

L174 The Zygomycota is no longer a recognized Phylum (now split into the Mucoromycota & Zoopagomycota; Spatafora et al. 2016)

Answer:

I changed it in the text.

Comment:

Figure 1. This is a matter of style but I feel the data could be presented in a different way that is more intuitive and impactful (e.g. boxplots?). The information is there, for me it just was not conveyed immediately

Answer:

I personally like this type of graph. I would like to keep it like that.

Comment:

Figure 2 I would be curious to see the N treatment treated as a covariate in an ANCOVA. Is the control slope steeper compared to the fertilized? Just looking at the plots it would appear so and may bolster the support for arguments made in the discussion about Ho and not Hi biomass that is contributing to SOM hydrophobicity.

Answer:

I am not sure what it means to use the N treatment as a covariate. A covariate is a continuous variable and the ANCOVA is used to test the effects of an independent categorical variable on a dependent continuous variable controlling for the effect of a second continuous variable (the covariate). I guess I can apply an ANCOVA for the data presented in Figure 2. Then I would be testing the effect of Fertilization on the Contact angle using the amount of new C as a covariate??

However, I am not sure that a covariate analysis will be the best for our data and hypothesis.

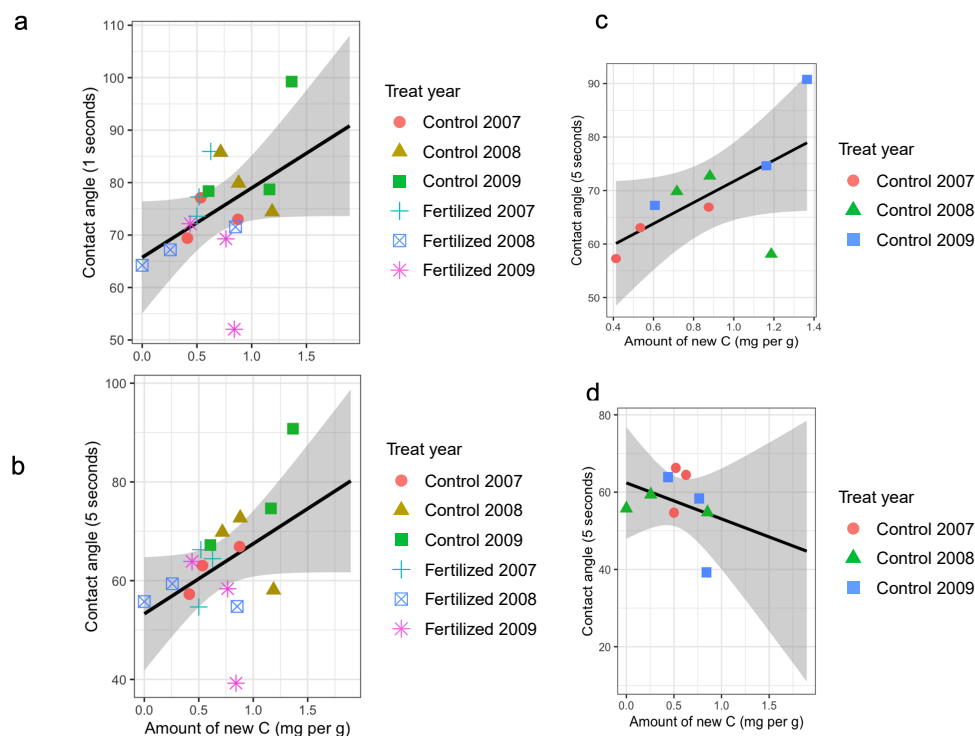
Generally, the covariate is not very relevant for the study question and the test is used to find if the differences between the treatments are due to initial selection differences (the covariate). So the test removes the variance between treatments caused by the covariate (Miller and Chapman, 2001 & Jamieson, 2004).

However, the amount of new C should not be used as a mere covariate since it is the main dependent variable that we try to use to explain the changes in hydrophobicity in time and fertilization regime. In this case the amount of new C might be intimately associated with the treatments (as we hypothesized) and removing its effect in an ANCOVA would remove most of the variance between the treatments (Miller and Chapman, 2001).

Jamieson, J. (2004). Analysis of covariance (ANCOVA) with difference scores. *International Journal of Psychophysiology*, 52(3), 277-283.

Miller, G. A., & Chapman, J. P. (2001). Misunderstanding analysis of covariance. *Journal of abnormal psychology*, 110(1), 40.

Talking about the slopes of the treatments, yes, the slope of the control is not only steeper but the correlation tends to be negative if the fertilized plots are analysed separately:



This make sense considering that the control and fertilized plots have opposite trends regarding hydrophobicity and as it is mentioned in the discussion; the changes in hydrophobicity cannot be explained only by the amount of hydrophobic EMF (being the new

C used as a proxy for EMF contribution). It depends on features specific of certain genera in this case *Piloderma* that was extremely scarce in the fertilized plots.

Additional information regarding this has been added in the results:

*There was a positive correlation between the amount of new C and the hydrophobicity for both CA1s and CA5s (Pearson, $T = 2$, $p=0.06$; $T = 1.9$, $p=0.07$; respectively) (Fig 2a and b respectively). **When breaking down the data by fertilization regime, there was a positive correlation between amount of new C and the hydrophobicity for the CA5s in the control plots (Fig 2c) but the correlation tended to be negative in the fertilized plots (Fig 2d)***

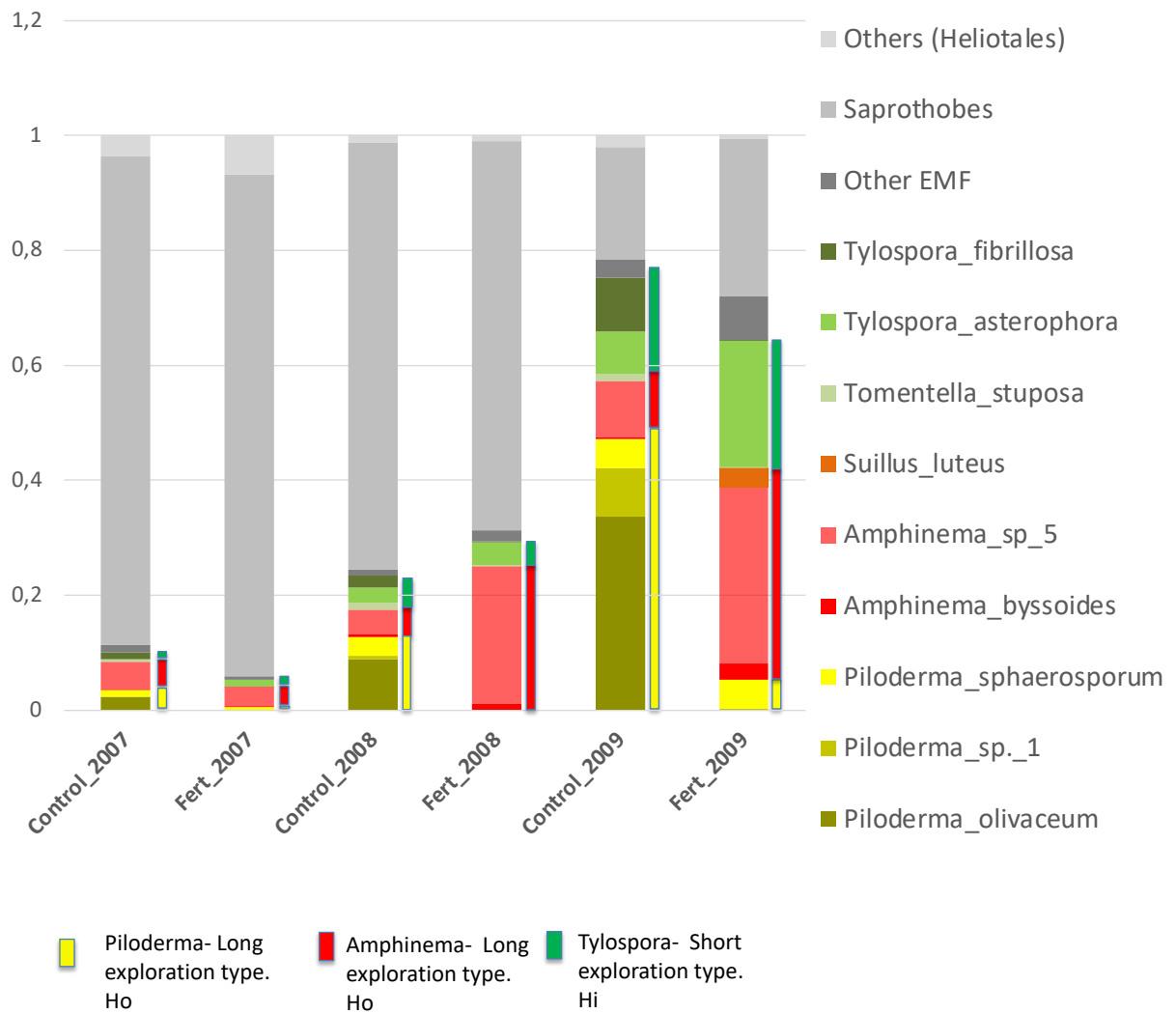
Also, those extra 2 figures were added as panel 2c and 2d in Figure 2.

Comment:

Figure 3b. Maybe in the key you could add the hydrophobicity of each of the taxa based on genus level classifications in Agerer 2001 and Lilleskov et al. 2011 for those that are not immediately familiar? Additionally, a third panel with the relative abundance of the two hydrophobicity groupings could be added.

Answer:

A new Figure 3 where the most abundant species (at least 1% of the total fungal reads) are grouped by genus and by exploration type and hydrophobic properties of their mycelium will be presented:



Also, in the same figure a boxplot of the most abundant genera will be shown:

