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Biogeosciences Discussions

Interactive comment on "Contributions of ectomycorrhizal fungal mats to forest soil respiration" *by* C. L. Phillips et al.

C. L. Phillips et al.

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Response to Referee 2

Referee comment: The manuscript reports findings from a multi-year field campaign of measuring soil CO2 efflux in a coniferous forest in the North-Western United States. Teh novelty in the approach is in the judicious placement of respiration sampling points, which allowed a pair-wise comparison of forest floor either with or without fungal mats formed by the ectomycorrhizal species Piloderma. This enabled a closer investigation of the flux contributions of such fungal mats, and the specific responses to environmental conditions. The approach has a lot going for it, as it avoids the necessary artefacts of using exclu- sion and in-growth approaches, as outlined in the introduction. Measuring in situ from existing mats removes the uncertainty of whether fungal abun-

dances have changed following e.g. trenching and mycorrhizal in-growth (e.g. using mesh approaches). The results illustrate that over an entire growing season, CO2 flux from forest floor areas with dense hyphal mats show higher fluxes than areas without mats, whilst temporal dynamics suggest considerable sensitivity of the fungal flux contributions to soil mois- ture.

Author reply: We value the referee's appreciation of our work, and are happy to see she or he was able to distill the key results.

Referee comment: What this approach assumes is that the occurrence of fungal mats at any place on the forest floor is random, i.e. that there is no underlying difference between mat and non-mat areas that determine the presence of the mat. I appreciate that these mats form over time, and may also recede from some areas, but underlying soil conditions may have an influence of whether a mat is found or not. This could at least in principle affect the validity of the approach, if underlying soil differences also affect soil CO2 production, and should be discussed in more detail. The use of paired control areas in close proximity makes sense, as it removes larger scale spatial heterogeneity, but soil heterogeneity on the forest floor is considerable at small scales (certainly below 1 m), and I miss a critical evaluation of this aspect.

Author reply: We agree with the referee that the validity of the approach depends on mat and non-mat areas not differing in other respects. Our third study question specifically tests this assumption, and is presented in the sections 3.3 and 4.2. In response to this and other referee's comments, we have expanded discussion of the study's limitations. One of the points we make is that mat colonization is influenced by 1) tree host, and 2) the abundance of downed wood in advanced stages of decay. Although we cannot rule out underlying soil conditions as a predictor of mat abundance, our spatial sampling regime did not reveal significant differences in moisture, texture, %C or %N in the underlying mineral soil to 35 cm depth below mat and non mat areas.

Referee comment: Notwithstanding this criticism, the results show interesting temporal dynamics (which are independent of the potential bias from paired comparisons). These dynamics are discussed in connection with soil abiotic conditions, particularly soil moisture. What has become quite clear in recent years is that root as well as mycorrhizal CO2 flux is strongly substrate dependent, i.e. belowground allocation of recent photosynthetic C may influence the amount of CO2 evolved from Piloderma mats as much as abiotic sol conditions. I appreciate that the experimental set-up does not include direct mea- surements of plant assimilation and/or belowground allocation of C, but this aspect should be included in the discussion. Soil moisture effects have clear impacts on plant assimilation fluxes, which may at least partly influence the observed flux responses.

Author reply: We have added this important point about moisture potentially impacting photosynthetic carbon supply to the discussion.

Referee comment: The inclusion of chitin degrading enzymes in the soil is interesting, as it highlights the turnover of fungal biomass in soils. The interpretation of these results has to be done more carefully, however, as there is some circularity in the argument. Clearly, higher abundance of chitin degrading enzymes results from the presence of mats in the first place. To explain hyphal respiration rates by the amount of chitin degrading enzymes does not make sense, as both are a result of the presence of mats, i.e. they must correlate, but the causation between them is not necessarily there.

Author reply: We absolutely agree that correlation does not imply causation, and our data, alone, are insufficient to demonstrate the cause of higher respiration in mat soils. We have changed wording to clearly distinguish the limited interpretation we can draw from our data, and hypotheses that might be posed from the body of literature on chitinase activity in fungal mats.

Referee comment: ECM fungi have been linked to soil priming, i.e. the decomposition of organic matter for obtaining nutrients (see e.g. Cheng and Kuzyakov work, or pa-

pers by Subke on mycorrhical C supply and priming). Clearly, these organisms have the capacity to utilise autotroph- derived C in form of root exudations whilst decomposing (or facilitating decomposition) of soil organic matter. Authors refer to rhizospheric respiration, rather than autotrophic respiration, in order to make clear that there is a continuum between plant roots and the C they provide to the rhizosphere, and the associated organisms that metabolise this exuded c as well as soil organic matter. The claim that EcM are generally regarded as “autotrophic” (line 397) is too simplistic, and this section (396-404) should be carefully re-written to account for present knowledge of rhizosphere C transfer and priming.

Author reply: We completely agree with the referee, and regret that we did not convey the state-of-knowledge on rhizospheric respiration. We ultimately removed that paragraph from the discussion.

Referee comment: Overall I think that the approach is interesting, and the estimate of temporal variations influxes as well as overall contributions are useful for comparison with other literature. As i point out above as well as in the detailed comments below, more work is required before the paper is publishable. The authors should discuss the relative merits of their approach more completely, and clarify several aspects (e.g. replication of profiles). Also the interpretation of results has to be done more carefully in places to avoid over- interpreting correlations as causally linked events (chitin degradation and respiration).

Author reply: We have included an expanded discussion on the study’s limitations, and have reworded the discussion of the chitinase correlation to not imply it provides evidence for causation.

Referee comments: L. 17: I’m not sure I understand what an “incremental increase” is. Sounds like a double-nomer, i.e. any increase is incremental. Consider omitting the word ”incremental”.

91, 92 & 93: Delete “incremental”.

287-291: Delete the word “increment” throughout the section.

Author reply: Our goal was to clearly distinguish “mat respiration,” i.e. the respiration rate of a mat soil, from “the difference between mat and neighboring non-mat respiration.” Mat respiration is not always higher than non-mat respiration, so just using “increase” would not work. We hope our edits our now clearer: we now simply the term “mat and non-mat difference”, and define it mathematically in the methods.

Referee comment: 64: Please update the Heinemeyer 2011 citation, which is now published as a full paper in Biogeosciences (in 2012).

Author reply: Done.

Referee comment: 89: I’m not sure you can assess causation with your approach. You can certainly obtain differences, and interpret them, but additional measurements would be required to establish causation.

Author reply: We are confused by this comment and unsure how to address it, as we cannot find any place in the text where we state an intent to assess causation.

Referee comment: 90-96: The research questions contain foregone conclusions – addressing questions 2 to 4 is only meaningful if the answer to question 1 is “yes”. As you clearly assume that there is a difference, I suggest you start with question 2.

Author reply: We have to politely disagree with the referee. We could not assume the answer was “yes” to the first question. In fact, mat respiration was not always greater than non-mat respiration. Sufficient variability in the difference was observed, however, to investigate environmental drivers.

Referee comment: 104: Delete Zeglin reference, unless this is now in print. 560-565: Only include papers that have been accepted for publication.

Author reply: Done. We regret this related paper on EcM mats from HJ Andrews was not far enough along in the publication process to cite in our manuscript.

Referee comments: 130: No comma after 50 145 (and throughout manuscript): Leave a space between numbers and units. 160: What are the dimensions of “schedule 40” PVC pipe?

Author reply: Done. We have addressed these comments and questions in the text.

Referee comments: 192-198: Please clarify how many such profiles you had. The error bars in Fig. 7 suggest true spatial replication for mat and non-mat locations – is that the case? Why aren’t there error bars on data in Fig. 8, however? In case there was no spatial replication, how reliable are the estimates?

Author reply: We have added text to the methods describing in words the subsurface sampling scheme shown in Fig. 2 (There were two CO2 profiles and one temp and moisture profile per area), and also detailing how we performed Monte Carlo simulations to produce the error bars in Fig. 7.

There are no error bars in Fig. 8 both for clarity, and because Fig. 8 is showing a regression analysis, and the errors for individual observations are not generally propagated to estimates of regression parameters.

Referee comments: 218-219: “Substrate quality” is a pretty ambiguous term, and not everyone would agree that C and N content sufficiently describe it (I for one wouldn’t). I suggest you state simply that you determined %C and %N of soil cores.

Author reply: Done. We removed “substrate quality.”

Referee comments: 246: Having an exponential moisture dependent is very unusual, and it is highly unlikely that soil CO2 efflux increases exponentially across the entire soil moisture spectrum. Unless you can show that this is the best model (compared to possibly a linear mois- ture response, or, more realistically, a saturation curve), I don’t see any justification for using it. As you present no results for the regressions described here, I wonder if it is necessary to include these. I don’t see what the advantage of doing these regressions is compared to taking direct estimates of pair-wise differences for mat fluxes from your data, and possibly fitting a regression through these. Using the difference of two re- gression curves strictly requires you to include a propagation of errors associated with each regression to derive Rm, which you avoid when you use direct estimates for flux differences for your analysis.

Author reply: We considered the approach the referee suggests in the model selection process, and ultimately decided multiple linear regression was a less complex solution than the alternative the referee proposes. We concede that if temperature is constant or not an important driver, a linear increase in soil CO2 efflux would be expected. However, we chose to characterize both temperature and moisture effects simultaneously within the same probabilistic model. The linear model we used (Eq. 3, and adapted for difference estimates as Eq.5) is far more simple than a generalized additive model combining a non-linear component for temperature response and a linear component for moisture. The more complex model seemed unnecessary for our purposes, because: 1) it would be less familiar to readers, 2) the simple linear model fit our observations well, and 3) our primary goal was to test whether or not moisture and temperature effects were significant, but not necessarily to scrutinize the form of those relationships. The model in Eq. 3 has been used frequently to describe soil respiration. For example we cite two studiesâĂŤone at HJ Andrews and another in WisconsinâĂŤthat contain examples of this model fit to multiyear datasets (Martin and Bolstad 2005, Sulzman et al. 2005). In fact, we expected this model to be familiar to many readers, which was expressly why we used it as a starting point to derive Eq.5. The purpose of Eq. 2-4 is to simply show how Eq. 5 derives from a commonly-used exponential moisture and temperature model of soil respiration.

Ultimately, we think our approach was very similar to what the referee suggests, except we fit a regression to the ratio of mat to non-mat respiration rather than to the difference between mats and non-mats. We examined the potential impact of estimating moisture impacts for several representations of mat and non-mat differences (figure attached). The p-values shown are for t-tests that the slope is different than zero. This shows

moisture effects were significant whether we represent mat respiration as a difference, a ratio, or ln(ratio).

Another reason we chose to analyze mat respiration increment as a ratio rather than an absolute difference was to be able to use linear regression tools. The absolute difference between mat and non-mat was sometimes negative, and we could not calculate logarithms of negative values to linearize an exponential relationship between respiration and temperature.

We also need to clarify that we did not fit two regression curves to derive Rm. Rm is simply the ratio of mat to non-mat respiration, and was obtained from direct observations. As shown in Eq. 5, we fit a single model to the Rm observations. The uncertainty of temperature and moisture effects, presented in Section 3.2, are the errors associated with these parameter estimates.

Our statistical methods were developed with guidance from statistical consultants from OSU Dept of Forest Ecosystems and Society.

Finally, it is important to clarify that the results of these regressions constitute the heart of our analysis, and the results are reported in Sections 3.1 and 3.2 and shown in Figs. 5 & 6. We regret it was not clear how the results linked to the statistical tests, and we have altered some of the language to try and clarify these linkages.

Referee comment: 281: “the mat” rather than “mat the”.

Author reply: Done.

291: 8% of what: average mat respiration, or soil respiration? It would be more meaningful to give the increment in terms of absolute flux, not as a %age.

Author reply: We have clarified that this is the percent difference between mat and non-mat respiration, which increases with soil moisture.

We politely disagree that absolute differences would be more meaningful. It is difficult to interpret the change in absolute flux without also knowing background flux rates under the same conditions. Furthermore, for the reasons described above, we feel the ratio of mat to non-mat fluxes is the best expression of mat and non-mat differences to use for statistical analyses. We explicitly state in the methods now that we use the ratio for statistical tests, and present the results as percent difference ([Ratio – 1]*100)

Referee comment: 292-293: This is not shown. Figure 7 does not contain soil moisture, and none of the regressions in Fig. 8 are significant. Please remove this claim.

Author reply: The referee is absolutely correct! We have reworded the statement accordingly.

Referee comment: 323: The p-value suggests no significant difference.

Author reply:

The referee is correct that chitinase activity was not different at the P= 0.05 level, and have added text to underscore this. Because the P-value was close to 0.05 level and other studies have found substantial difference in chitinase activity between mat and non-mat soils, we feel it is still relevant to present this result.

Referee comment: 332-336: This calculation is highly skewed, as it bizarrely regards soils underneath tree trunks as not part of the ecosystem, and having no relevant soil CO2 efflux. Surely the contribution of mats has to relate to total soil area, and if part of the soil surface is covered by fallen trees, then the proportion of mats is still responsible for the same amount of surface flux. Mat respiration has top be multiplied by its true areal extent, which is 42%, not 56%. Please correct this.

Author reply: We regret that we did not completely explain our reasoning for presenting mat cover in two different ways. We certainly do not have the view that soils underneath tree trunks are not part of the ecosystem; however, we also have no way of knowing what respiration rates are in those areas, and we feel we cannot assume they are similar to non-mat areas.

We have edited this section to indicate our upscaling estimates for mat respiratory contributions apply only to the exposed part of the forest floor where we characterized respiration rates.

440: the references are incomplete.

Referee comment: Figure 5: The regression lines appear linear, despite your data analysis indicating exponential response functions. Please make this consistent. Explain regression lines in the legend, and give regression fits and functions.

Author reply: The regression lines are linear because the y-axis is ln-transformed.

We have added to the legend an explanation of the regression lines, the significance of the slopes, and referred the reader to the functions in the methods. It is not customary to report goodness-of-fit (R2) for mixed effects models.

Referee comments: Figure 6: What is the regression response? Is this a significant line? Figure 4 shows gravimetric O-horizon moisture of up to 300%, which doesn't match your x-axis here. Please clarify. Why is the logarithm of respiration shown, rather than respiration directly?

Author reply: The logarithm was shown in Fig. 6 to be consistent with the linear regression model (Eq. 5); however, we now exponentiated the results to present on a natural scale, and added the p-value for the slope.

We accidentally plotted % water content rather than gravimetric water content, but have now fixed that error.

Figure 8: Where regressions are not significant, you should not include lines (i.e. top two panels), as these suggests trends that have not truly been detected. For the O horizon at least, it would be useful to show mat and non-mat locations with different symbols (open vs. filled circles).

Author reply: We made all these changes the reviewer suggested.

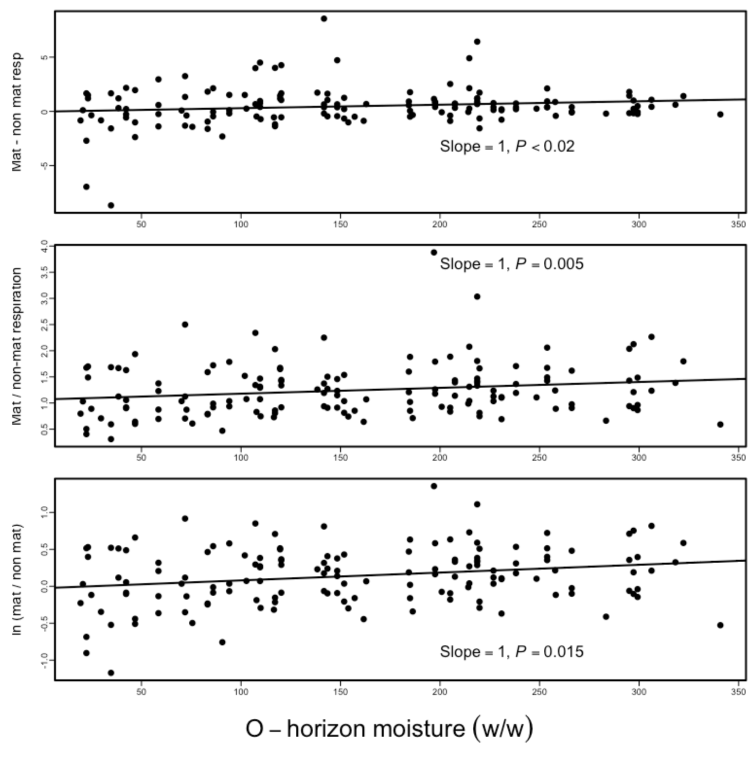
Mat - non mat resp
Slope = 1, P < 0.02
Mat / non-mat respiration
Slope = 1, P = 0.005
ln (mat / non mat)
Slope = 1, P = 0.015
O – horizon moisture (w/w)

Fig. 1.