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Karen Haubensak, Ph.D.

Subject-matter Editor

*Ecosphere*

Dear Dr. Haubensak,

We are pleased to submit a revised version of *Ecosphere* manuscript ECS18-0757, “Genetic variation in tree leaf chemistry predicts the abundance and activity of autotrophic soil microorganisms”. We have fully addressed each of the three reviewers’ comments in detail below. Our responses immediately follow each reviewer comment, page and line numbers listed in our responses refer to the revised version of the manuscript.

Consistent with ESA’s data policy, we have made our data and R code publicly available at:

https://www.github.com/selmants/CT\_AmoA

We expect our paper to be of immediate interest to a wide audience of microbial ecologists, ecosystem scientists, and evolutionary ecologists. We hope you find our revisions and responses to reviewers’ comments sufficient to merit publication of our manuscript in *Ecosphere*.

Sincerely,

Paul C. Selmants, Ph.D.

Reviewer #1 (Comments to the Author):

This is well-written and interesting manuscript from a research group that has done foundational work in this field from the late nineties. Their current findings fit well to the earlier research done in their study system and broadens our understanding of the effects of plant genetic variation in ecosystem functioning. I have only few questions and suggestions throughout, yet I also noted something incoherent especially in statistics/statistical tests. See line-by-line comments below.

Line 155-156: The amplification efficiency seems to be quite low. Ideally, it should be over 95%. I just wonder why did you choose these particular primers as the efficiency is not in ideal range?

Line 157, Data analysis paragraph: Maybe it’s better to write about ANOVA tests first and thereafter the regressions (then it follows the same order as in results section)?

Line 161-162: Not sure, but should it be “nitrification potential” instead of “potential nitrification”?

Line 164-165: Why did you used higher than normal significance level as in your (significant) correlations p is below 0.03?

Line 168-169: I don’t understand why significance level is set to “unnormal” 0.10 as the effect of tree zone on the ratio of archaeal-to-bacterial amoA was not even near to significant. If I understood right, you have 6 soil samples per 3 stands within each zone. I think that it should be enough to demonstrate significant effects of tree zone (if there are such) even if you use a “normal” 0.05 significance level.

Results:

Line 172: The first part of this sentence belongs to discussion. I think that you can start directly from “Foliar CT concentrations…”

Lines 173-175: You should also present statistical tests here (ANOVA results), right? Now, only the means are presented, but their statistical difference is not tested anywhere.

Line 183: “because of the high within-zone variability”, can you write like this if you don’t present how high the within-zone variability was? In my opinion, you should give e.g. a range within each zone or something else (supplementary figure?) to describe this data in more detail.

Fig 1. Could you separate the observations from different zones in these figs? E.g. open circle for Fremont, closed for Narrowleaf and grey for hybrids etc. It’s quite easy to imagine which are from Fremont, but it would be interesting to see how narrowleaf and hybrids are located in these regression lines.

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Reviewer #2 (Comments to the Author):

The paper by Selmants et al. examines the variation and links of leaf condensed tannin (CTs) concentrations, soil nitrification rates and abundance of bacterial and archaeal ammonia oxidizers among stands of two Populus species and their hybrids.

The paper is clear and easy to read, but very shallow in terms of providing new information. Variation of leaf CT concentrations among Populus species and their hybrids and the influence of this variation on soil N transformations have been shown in numerous studies, which leaves the correlative link between leaf CT concentrations and soil bacterial and archaeal oxidizer abundances as the only novel aspect of this study. That the variation of abundance of archaeal oxidizers, but not that of bacterial oxidizers, seems to be linked to the variation of soil nitrification rates among the field sites is an interesting observation as such. However, this link is correlative and in fact, depends heavily on one Fremont site of high archaea abundance. Is such observation worth of publication or should it rather be used as a starting point for experiments that corroborate the finding and reveal the mechanisms behind the observation? Looking at Fig. 1, it seems that the observed trends between bacterial and archaeal ammonia oxidizers and leaf CT concentrations are due to Fremont sites having significantly less bacterial than archaeal ammonia oxidizers, while no big differences appear in other sites. With this data set, isn't it premature to conclude that leaf CT concentrations drive the abundance of archaeal, but not those of bacterial ammonia oxidizers? What if the Fremont sites simply provide a great habitat for archaeal, but not for bacterial ammonia oxidizers and there is no real link between leaf CT concentrations and ammonia oxidizers? I would like to see more work on the subject to support the conclusions before publication.

Given that the title includes "genetic variation" and much of the text is built on this concept, there is little evidence of intraspecific variation in nitrification rates or bacterial and archaeal abundances (Fig. 1). The results suggest that the two species are different and have different effects on soil N transformations, but there is no genetic variation within species (not even the hybrids differ from both parent species). In fact, the reader remains puzzled how intraspecific genetic variation could be tested with the sampling scheme used.

As a minor point, the values of leaf CT concentrations given in the first sentence of results and in Fig. 1 do not seem to fit together.

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Reviewer #3 (Comments to the Author):

The authors present a well-thought-out study that simply addresses a gap in the literature and establishes a potential mechanism subject to selection whereby plants can engineer soil autotroph microbial communities. The manuscript is well-written and does not require significant modification; however, I do believe that the authors need to further justify their statistical interpretation of their results. I understand the potential necessity for adjusting the significance threshold from 0.05 to 0.1, but in so doing they invite questioning of their results. This is problematic in the context of Figure 1B as it makes one wonder how much of the significance of the depicted relationship could be potentially do to one outlier site where high abundances of AOA gene copies were observed. It may be worth addressing this concern in the text in order to add strength to the author’s interpretation of their results by addressing a potential source of skepticism. I also feel as though the authors have overstated the gradient aspect of their study a bit, as their distribution of sites ends up being somewhat bimodal, especially when looking at the trait they are most interested in determining the impact of (foliar CT concentrations). This is something that should be admitted to in the discussion as a potential pitfall of the design. It does not downplay the effect of their results, however, as they show that hybridization therefore likely does have a significant effect on the traits they measure. Other than those concerns, I find the manuscript acceptable for publication with minor specific comments as follows:

64 – I believe a comma would help this sentence (“previously unknown, large and nearly ubiquitous”).

89-92 – This addendum is not necessary here, you can add what is novel here to the similar segment at beginning of paragraph or just remove this sentence entirely.

106 – The term “narrowleaf” has not been defined yet and its meaning must be inferred.

115 – I might quibble with calling “putting on dry ice” “flash-freezing.”

129 – Was soil moisture measured as well? If so it should be included, or reasoning should be provided as to why it was not a relevant parameter.

142 – You do not have to do so here, but either here or in the discussion some of the pitfalls of using qPCR, particularly to infer function, should be mentioned. That can also be a way in which you assuage any concerns readers might have about the variability in amoA results observed in some of the particularly low CF sites.

169 – Was there a reason why you still used a cutoff of α = 0.1 when you have more replicates to work with for this set as a result of the individual soil sample being your unit of replication?

250 – The “although” in this sentence is not needed.

Table 1 – Why doesn’t this table include the foliar CT concentrations observed? If those methods are detailed then the results should be explicit by treatment group somewhere in the text.