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Interactive comment on "Soil microbial communities following bush removal in a Namibian savanna" *by* J. S. Buyer et al.

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We thank Referee #3 for his/her careful criticism of the paper. We would like to respond to the major criticisms of the experimental design and then the specific criticisms.

Referee: However, the design of the experiment that, in my opinion, is not valid to reach the aims planned in this study. If the authors intended to assess whether the changes promoted by a invasive plant on soil microbial communities diminish or disappear after its management using the thinning, they should have selected a noninvaded area as control of the original state of savanna. Bush encroachment is a major disturbance to the ecosystem and the recovery of soil microbial community after bush thinning should be referred to pre-invasion conditions.

Authors: As stated in the paper, our hypotheses were (1) In a savanna ecosystem soil microbial community structure is different under grass than under woody plants, and (2) the soil microbial community is resilient to the disturbance caused by bush thinning. The experimental design, using control plots that had been invaded but not thinned, was adequate to test these hypotheses. If we had been testing the hypothesis that thinning restores the ecosystem to a pre-invasion state, then the non-invaded plot would have been essential as a control. In fact, we completely agree with the reviewer that this would have been ideal. However, it was impossible as there were no non-invaded areas near the study site. The only non-invaded areas were so far from the study site that spatial variance would have rendered those areas useless as controls. We were very careful to state that we were measuring recovery from the disturbance caused by bush thinning, not recovery from bush invasion, and not recovery to a pre-invasion condition. This is, we agree, not ideal, but it does provide an experimental framework to test our stated hypothesis.

Referee: Another concern is the lack of replicates of each treatment; as only one plot by treatment was performed. Authors indicated in Statistical analysis section that the factor thinning was not pseudoreplicated because there were 3 pairs of thinned and control plots. However, the 3 thinned plots correspond to the three levels of factor thinning since each plot has a different time of thinning. In my opinion, only vegetation factor was replicated.

Authors: We worked very closely with a highly qualified statistician to make sure that our analysis was valid. With 3 pairs of thinned and control plots, and using a mixed model of variance to account for spatial and temporal variation, both factors (thinning and vegetation) were not pseudoreplicated. Any effect of the time of thinning would have been included in the spatial component of variance since there was one plot for each year of thinning. However, if we had tried to determine the effect of time of thinning in this model, using time of thinning as 3 levels as you describe, the analysis would indeed have been pseudoreplicated and entirely invalid. We would also like to

point out that pseudoreplication is not always a straightforward concept to apply. In some cases it is a matter of judgment as to whether an experiment is pseudoreplicated or not, and whether a pseudoreplicated experiment is still useful or not. There has been much discussion of this in recent years, and here are two references on this point:

Heffner, R.A., Butler, M.J., Reilly, C.K., 1996. Pseudoreplication Revisited. Ecology 77, 2558-2562.

Schank, J.C., Koehnle, T.J. 2009. Pseudoreplication is a Pseudoproblem. J. Comparative Psychology 123, 421-433.

As Schank and Koehnle stated in their paper cited above, "The problem of pseudoreplication rests on the question of whether data gathered with any degree of spatiotemporal proximity is too intercorrelated and statistically interdependent to permit statistical inference." In our case the use of a mixed model of variance permits us to test hypotheses regarding thinning and vegetation without pseudoreplication, but not a hypothesis regarding time of thinning. Instead, the effect of time of thinning was inferred but could not be separated entirely from possible effects of spatial variation, as we clearly stated in section 3.1.3 and in the last paragraph of section 3.2.2.

Referee: If the Journal considers acceptable the use of pseudo-replicates, the authors should perform a statistic analysis of the data in Tables 1 and 2. The effect of treatment thinning (thinned vs. control plot) was not statistically analysed and then it cannot be concluded if soil chemistry and PLFA concentrations were more affected by the type of vegetation or by the treatment thinning.

Authors: Table 1 was statistically analyzed because, as we stated above, the effect of thinning was not pseudoreplicated. The statistical analysis was clearly presented in Table 1. Table 2 was not and should not be statistically analyzed because the effect of time of thinning is indeed pseudoreplicated and therefore any statistical analysis would be invalid. We believe that Table 2 contains useful information, particularly when combined with the microbial community analysis presented in Figures 3-6, that should be

presented despite the lack of statistical analysis. As Hurlbert said in the original paper on pseudoreplication (Hurlbert, S.H., 1984, Ecological Monographs 54, 187-211), "Because an obsessive preoccupation with quantification sometimes coincides, in a reviewer or editor, with a blindness to pseudoreplication, it is often easier to get a paper published if one uses erroneous statistical analysis than if one uses no statistical analysis at all." We believe that we have avoided this trap, providing a statistical analysis where it is valid and avoiding it where pseudoreplication renders such an analysis invalid. Most importantly, we clearly stated which data was pseudoreplicated, and we were extremely cautious in our interpretation of it, so the issue was clearly laid out and the reader was not deceived.

Referee: Specific comments: -The application of PLFA 16:1ω5 as biomarker of AMF is limited due to its presence in bacteria (Frostegård et al. (2011). Soil Biology and Biochemistry 43, 1621–1625.

Authors: We completely agree with the referee on this point. All of the PLFA biomarkers need to be interpreted very cautiously. We will add a statement to this effect in the revised manuscript along with the suggested reference.

Referee: What month was carried out the thinning? How many times were the plots thinned each time?

Authors: Each plot was thinned once. We will add more information on the exact time of each thinning in the revised manuscript.

Interactive comment on SOIL Discuss., 2, 1393, 2015.