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Biogeosciences Discussions

Interactive comment on "Increased physical protection of soil carbon in the mineral soil of a poplar plantation after five years of free atmospheric CO_2 enrichment (FACE)" *by* M. R. Hoosbeek et al.

Anonymous Referee #2

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This study examines the effect of atmospheric CO2 enrichment, N fertilization, and poplar species on (1) the size distribution and C and N contents of water-stable soil aggregates, (2) the formation of microaggregates within macroaggregates and (3) the amount of C and N associated with the microaggregates isolated from within macroaggregates. The study was conducted at the EuroFACE short rotation poplar plantation after five years of CO2 enrichment, which was two years after the first coppicing. Nitrogen fertilizer was added only in the fourth and fifth years. Because an increase in bulk soil C and labile C were reported after five years of CO2 enrichment (Hoosbeek et al.,

2006, Plant and Soil 281:247-254), the authors hypothesized that this extra labile soil C would increase the formation of macroaggregates and, subsequently, the formation of microaggregates within macroaggregates. The focus of the study and its primary hypothesis are important and timely topics - both because of concerns over rising atmospheric CO2 concentrations and the need to better understand the mechanisms controlling the cycling and potential stabilization of organic matter in soils, particularly under various management practices designed to increase C inputs to soils (e.g., N fertilization, restoration of cropland to perennial species). However, there are two major issues with the manuscript that need to be addressed. One concerns the statistical analyses and the other is methodological. Both could significantly affect the interpretation of the results and the conclusions of the study.

With regards to the statistics, there are problems with the assignment of replicates within the experimental analysis. The experimental design appears to be a split-split-plot; i.e., the CO2 treatments are the main plots, which have been split into the two N fertilization treatments, and these subplots are then further split into the three sub-subplots with different poplar species. The ANOVA for a split-split-plot design would still enable analysis of the main effects of CO2 treatment, N fertilization, and species but would also permit evaluation of potential interactions between these treatments. Although not examined by the current analyses, potential interaction effects are certainly worthy of investigation. In split-split-plot analysis, the degrees of freedom are more properly assigned to the treatments and errors, thus avoiding the pseudoreplication present in the current analyses. For example, in the analyses presented in the manuscript, each of the CO2 treatments is given a sample size of 12 (2 replicate CO2 treatment plots x 2 N fertilization treatments x 3 species). This is improper; the experimental unit is the CO2 treatment plot, of which there are only 2 replicates. The 6 samples within each plot are not true replicates; they can only be averaged to give the best estimated value for each plot. The authors should consider consulting with a statistician and employing a split-split-plot design in their analysis, but if they insist on analyzing each experimental variable in separate one-way ANOVAs, then the proper

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sample sizes must be assigned (2 per CO2 treatment, 4 per N treatment, and 4 per species).

The concern with methodology has to do with the isolation of microaggregates from within macroaggregates. The use of the Six et al. (2002) microaggregate isolator is state-of-the-art. However, with this device any fine (53-250 micron) inter-microaggregate particulate organic matter (POM) released through the dispersion of macroaggregates or unstable microaggregates will be captured on the 53-micron sieve with the intact microaggregates. Unless steps are taken to remove this released POM, which is NOT occluded within the microaggregates (e.g., by density flotation), the C content of the fraction caught on the sieve does not truly represent C "protected" within microaggregates. Because the weight of this released POM is generally insignificant compared to that of the microaggregates, changes in the amount of microaggregates can usually be assessed without removing the released POM, but the C and N contents must be carefully evaluated. In some soils, the amount of this material might be small enough to have little effect, but this should be demonstrated before the C contents of this fraction are claimed to be "protected" within microaggregates. Similarly, the presence of free or released POM caught on the sieves during standard wet sieving of bulk soil can erroneously inflate the C content assumed to be occluded within macro- and microaggregates, if not removed. The authors also need to clarify whether or not the weights and C and N contents of the iM-microaggregates (Table 5) were sand-corrected in the same manner as the wet-sieved fractions. One could assume that they were on the basis of lines 19-20 on page 878, but the table does not explicitly say that the data are sand-corrected as was stated in the titles of Tables 2-4.

The following is a list of more specific comments and corrections:

p. 875, line 19: Clarify what is meant by "the condition of minimum CO2 enrichment pollution".

p. 875, line 21: Suggest changing "nitrogen differential treatments" to "differential ni-

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trogen treatments".

p. 875, lines 23-27: Please clarify. Were the 3 different poplar genotypes inter-planted with the genotype planted across all 9 ha or in place of this genotype at a higher density?

p. 876: The methods section needs more detail on soil sampling and analysis. How were samples collected - by coring? How many samples were taken within each sub-plot; if more than one, were they composited for analysis? Were the samples collected for bulk density also used for bulk C and N analyses? Were the roots removed from the soil before or after drying? Were carbonates present in the soil? If they were present, were they removed before elemental analysis?

p. 876, line 26: Six et al., 1998 manually wet sieved soil sequentially through a series of sieves, and thus this paper is not really an appropriate reference for wet sieving with stacked sieves, especially since it appears that a mechanical apparatus was used in the current study. Sequential sieving usual subjects increasingly smaller aggregates to greater disruptive energies than one-time sieving with a set of stacked sieves. Thus, it would be more appropriate to cite a reference that also used stacked sieves.

p.878, line 7: Change "en" to "and".

p. 878, line 10: Change "course POM and sand" to "coarse POM plus sand".

p. 878, lines 19-20 and Table 5: Please clarify whether the C and N fractions for the iM-microaggregates are based on the g C in the total bulk sample or the g C in the combined small plus medium macroaggregate fraction that was dispersed by the microaggregate isolator. If it is based on the g C in the total bulk sample, then mass balance suggests that essentially all of the C in this combined macroaggregate fraction ends up in the iM-microaggregates for most treatments. Furthermore, for the FACE treatment, there would then be even more C in the iM-microaggregates than in the combined macroaggregates, which is impossible except for analytical errors.

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p. 880, line21: “Few earthworms” implies hardly any. If what is really meant is that some earthworms, but not many, were observed, then it would be better to state “a few earthworms” or “limited numbers of earthworms”.

p. 881, line 6: Should explicitly state “increased the formation of free microaggregates”.

p.881: More free microaggregates could also result because the macroaggregates that were formed under P. euramericana are not as stable as under the other species and they break up during wet sieving. This could happen even though P. euramericana had higher root C inputs if, for example, it has coarser roots (shorter root lengths) or less associated mycorrhizal fungal mycelium to help stabilize the aggregates.

Table 1: Were statistics run for these data or were there simply no “significant” responses?

Interactive comment on Biogeosciences Discuss., 3, 871, 2006.