

Interactive comment on “Increased physical protection of soil carbon in the mineral soil of a poplar plantation after five years of free atmospheric CO₂ enrichment (FACE)” by M. R. Hoosbeek et al.

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We would like to thank referee #2, and prof. G. Pan for providing interesting and useful comments. Comments made by referee #2 with respect to the statistical method affected the outcome of our study (see below). The means of the measured variables did of course not change, but several CO₂ treatment effects were no longer significant ($P < 0.05$). Because of this we changed the title of our revised manuscript.

Referee #2 raised two major issues and provided a series of more specific comments. The first issue concerns the statistical method we used. Referee #2 correctly points

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out that the experimental design is a split-split-plot design, i.e. the CO₂ treatments are the whole-plots, which have been split into two N fertilization treatments, and these sub-plots are then further split into three sub-sub-plots with different poplar species. We took soil samples from each sub-sub-plot at a random location within the sub-sub-plots. We therefore considered the six samples from each main-plot to be independent from each other. As suggested by referee #2 we consulted a statistician. Dr. Evert-Jan Bakker (Mathematical and Statistical Methods Group, Wageningen University) confirmed, from a strict statistical point of view, that the unit of replication for the CO₂ treatment is the whole-plot. With concern to the CO₂ treatment, the six samples should be averaged per whole-plot. This, of course, seriously limits the statistical power with respect to the CO₂ treatment.

At the inception of the POPFACE/EuroFACE experiment, the whole-plots (3 ambient and 3 elevated CO₂ rings) were assigned randomly within the poplar plantation. In order to account for this, Dr Bakker advised us to include a random factor “location” in our statistical model. Location was defined as the whole-plot (ring) number (RingNr) within the CO₂ treatment. Based on the split-split-plot field design we used the following univariate general linear model (SPSS 12.0.1): CO₂trmt (fixed) Ntrmt (fixed) Species (fixed) RingNr(CO₂trmt) (random) CO₂trmt*Ntrmt Ntrmt*RingNr(CO₂trmt) CO₂trmt*Species Ntrmt*Species CO₂trmt*Ntrmt*Species

For practical reasons, i.e. limiting the amount of lab work, we used samples from 4 whole-plots (ambient rings 2 and 3; FACE rings 1 and 4). The number of replicates per treatment are: CO₂ trmt n = 4 (2 ambient + 2 FACE) N trmt n = 8 (4 unfertilized + 4 N-fertilized) Species n = 24 (8 *P. alba* + 8 *P. nigra* + 8 *P. euramericana*)

The results of applying this revised statistical model to our data are included in the revised manuscript.

The second issue addressed by referee #2 concerns the isolation of micro-aggregates from within macro-aggregates. Indeed, next to the micro-aggregates, a small amount of

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fine (53-250 micron) inter-micro-aggregate particulate organic matter (POM) released through the dispersion of macro-aggregates or unstable micro-aggregates may have been captured on the 53-micron sieve. We assumed this POM material to be negligible as compared to the isolated micro-aggregate fraction, especially on weight basis. But, we agree that the C and N contents may have been affected to some extent. Not all of it may have been “protected”. We address this issue in the discussion section of our revised manuscript.

The iM-micro-aggregate fractions were sand-corrected in the same manner as the wet-sieved fractions.

P 875, line 19: A minimum distance of 120 m between FACE and control (ambient) rings was kept to prevent CO₂ pollution from FACE rings to control rings. “randomly assigned under the condition of a minimum distance between plots of 120 m to avoid CO₂ cross-contamination.”

P 875, line 21: OK.

P 875, lines 23-27: The 3 different poplar genotypes were planted at a higher density in place of the genotype planted across all 9 ha. We added: “These six plots were planted at a density of 1 tree per m² using three different genotypes.”

P 876. OK, we answered these questions by extending the text of the manuscript (paragraphs 2.0 and 2.1).

P 876, line 26: OK.

P 878, line 7: OK.

P 878, line 10: OK (indeed, makes it more clear that “course POM plus sand” is one fraction).

P 878, lines 19-20: The sentence “Weight, C and N fractions were calculated as mentioned above.” was not correct. The iM fractions (table 5) are not based on the total

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sample weight, but on the 250 - 2000 μm macro-aggregate fraction. We defined how the iM fractions were calculated.

P 880, line 21: “some earthworms” is what we observed. So, we changed the text to “a few”.

P 881, line 6: OK.

P 881: This explanation may be possible as well, although the C input due to *P. eu-ramericana* did not have relatively more coarse roots or less associated mycorrhizal fungi (Lukac et al., 2003). Therefore, we render this explanation less likely to be true than explanation 2 as mentioned in our manuscript (speeding up of cycle of aggregate formation under *P. euramericana*).

Table 1: No statistics were run on these data. These are just the soil data for the samples collected in October of 2003. Because initial soil data were different per ring, only the change between the initial condition and the state after for instance 5 years would be meaningful for statistical analyses (as was done by Hoosbeek (2006)).

We thank prof. G. Pan for his positive comments. As mentioned by prof. G. Pan, many researchers observed new soil C to be in a relatively free state (free POM or in large macro-aggregates). The fact that we observed a species effect on micro-aggregates after five growing seasons was also a surprise to us. Also, within five years FACE treatment enhanced the C content of the micro-aggregates within macro-aggregates, although with the current statistical model this difference is not significant anymore. Still, the fact that the formation of micro-aggregates is affected within five years by species (or treatment) shows that the turn-over rate of the model of aggregate formation is relatively fast. Faster than we thought. At least some of the new C that entered the soil during the experiment must have been incorporated in newly formed micro-aggregates. We agree that future work with isotopes would be helpful in gaining insight into the processes that facilitate the protection of C in the soil.

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Sincerely, Marcel R. Hoosbeek

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