Duvert et al

Duvert et al. surveyed agricultural and forested headwater watersheds in tropical Australia to address the question: 'How does land use change and flow conditions affect the age of DOC exported from these watersheds? Hypotheses to be tested are:

- H1- land use conversion to agriculture leads to the export of older DOC because erosion has depleted modern soil OC
- H2- younger DOC is exported during high flow conditions compared to baseflow because high flows mobilize younger OC from shallower soil layers.
- H3- stream DIC is younger than DOC because DIC is derived from root respiration and decomposition of younger, more labile DOC (i.e., DIC and DOC ages are uncoupled).

As the authors note, the tropics are understudied with respect to carbon loss in general, and in response to agricultural activities in particular. Thus, this is a valuable question to explore.

The cost of analysis means that sample sizes are understandably small for studies based on 14C data. The authors have supplemented their 14C-DOC results with other measures of DOC quality, but in the end, they are still constrained by a small sample size (N = 18 and 16 for their dry and wet season datasets, respectively - or 6 and 12 forest and agricultural sites).

The key set of results are presented in Fig. 2, with statistical results of comparisons between land use types for a given season, and seasonal effects within each land use category. There is a fair amount of variability in the results, thus the authors used generalised additive models (GAMs) informed by 9 driver variables to identify the sources of this variability. This choice is problematic, as GAMs and other ML approaches are built for use with large data sets. The effective degrees of freedom for non-linear relationships GAMs tend to be high. A common rule of thumb for various (less complicated) regressions is 10 observations per parameter (Harrell 2015), suggesting that an appropriate sample size for 9 covariates would be well into the hundreds and beyond. Thus, I am skeptical of the robustness of results presented in Fig. 4 and wondered if confidence intervals were excluded from these plots because they were large. The authors should give serious consideration to removing this analysis from the manuscript.

It took me a while to sort through the results and discussion, perhaps not surprising given the fact that I do not work with isotope data on a regular basis combined with the consideration of 9 different response variables across two land uses x two seasons, and the reality that interpretation of isotopic data can be tricky because multiple sources and processes may shape these values. Unfortunately, this complexity was enhanced by occasional contradictory or slightly misleading statements and introduction of new results in the Discussion (see line-specific comments for examples). Figure 6 is a very nice way to summarize the authors' hypothesis for the differences in DOC age shown in Fig. 2g. It makes sense, but there are some missing or ambiguous pieces of evidence in its support- mostly for the agricultural side of the figure, and at times it felt as if the authors were working hard to make their data fit into this model alone. The most obvious evidence gap is the assumption that soil C in agricultural areas is old at all depths. Soil erosion is a reasonable mechanism for moving soil C to the stream in agricultural streams, but evidence that this is happening is lacking (see comment for line 290). As the Duvert et al. acknowledge, their rainforest sites are steeper and they also tend to occur at higher elevations than agricultural sites. Agricultural areas are often former grasslands, which have distinct structure and carbon storage compared to forests. This leads to the question: is it agricultural disturbance that is creating the differences reported here, or is it something to do with these differences in physical attributes and/or a legacy of past land cover?

Regardless of mechanism, the age differences shown in Fig. 2g and the finding that DOC in these sites is unexpectedly old are intriguing results. Because I am a non-regular user of 14C data, I was interested in having some context for the old DOC in these streams. This led me to Table 1 in Shi et al. (2020). Put another way, is there any contextual data available for considering DOC ages presented here?

Line 46- It seems like the recent paper by Dean et al. (2025) should be acknowledged/incorporated into this paragraph.

Line 71- 'The humid tropics of Australia is a mountainous region…

Line 226-228- This sentence is confusing- it starts by stating that there were no differences for streams draining the same land use category (i.e., within land use type), but then it goes on 'with no differences between land use categories…'.

Also, here you report that there is no significant difference between land uses during the wet season ($p = 0.073$). In other locations in the paper, p values slightly greater than 0.05 are noted as indicating differences (e.g., lines 222, 230, 231, and in a slightly different fashion, line 291). Given the small sample size in this study, I think some leniency on p values would be appropriate. Consider adding some text to the methods such as '$p < 0.10$ were viewed as being indicative of differences, given the small sample size.' But whether or not this is done, there needs to be some consistency in how these statistical results are reported.

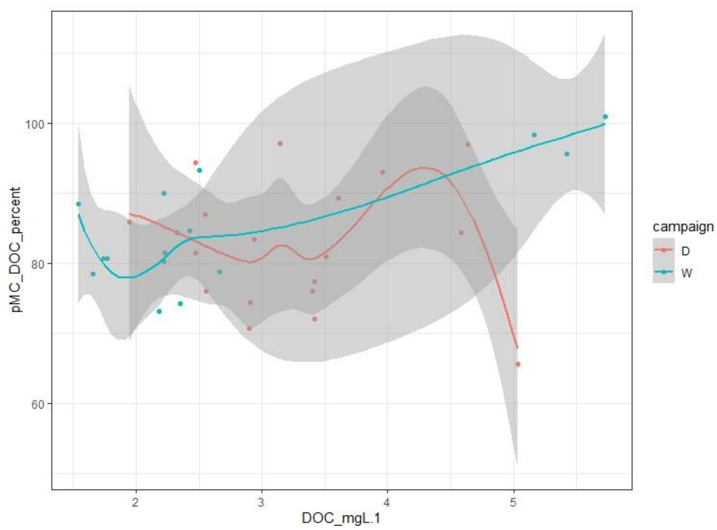
Line 230-231- The statement that "in agricultural catchments, high flows tended to mobilise DOC of similar age or older compared to dry season flows ($p=0.129$)" feels a bit misleading. Fig 2g shows samples with wet season DOC ages that are both older and younger than some of the DOC collected during the dry season. By itself, this figure indicates that the age of DOC in agricultural streams did not differ between seasons. And with respect to the comment about p values above, this one seems a bit too high to hint at a meaningful environmental difference.

Line 231-232- I am also confused by '8 of 10 sites with repeated measures' here. Weren't all 12 agricultural streams sampled twice, per lines 119-120? Can you clarify?

Fig 4 (if it is retained)- Please explain the barely visible dashes on the upper and lower horizontal axes. Typically, these dashes indicate the number of samples at each predictor value, and I first assumed that 1 set of dashes (e.g., on the top of each plot) represented predictor values during one season/model, and the second set (along the bottom) corresponded to the other season/model. However, the distribution of predictor values on top and bottom look the same. If these dashes are to be included, then they need some explanation. These dashes can be useful, particularly given that the ‘curves’ do not have any confidence intervals. I worry that the confidence intervals were omitted because the small sample size led to wide confidence intervals, indicative of a weak model.

Line 258- unclear what is meant by ‘DIC and DOC ages varied consistently across seasons’ given that changes in DOC ages were in opposite direction between seasons.

Line 280- Line 243 states that pMC-DOC was positively correlated with DOC concentration in the dry season, per Fig. 4. But there is no similar statement about a negative effect of DOC concentration on DOC age during the wet season though, which is not surprising given that panel 4e suggests that this relationship is fairly weak. Thus, this statement is new news. Table S2 (which is mentioned only in the Methods section) does show a significant relationship between DOC concentration and age during the wet season, but does not indicate the direction of this relationship. I was curious, so I plotted the data with a loess smoother (+/1 1SE):

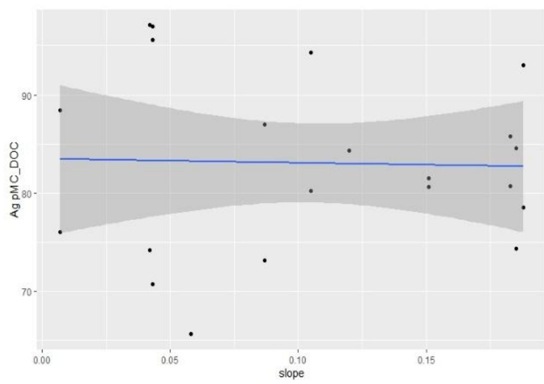


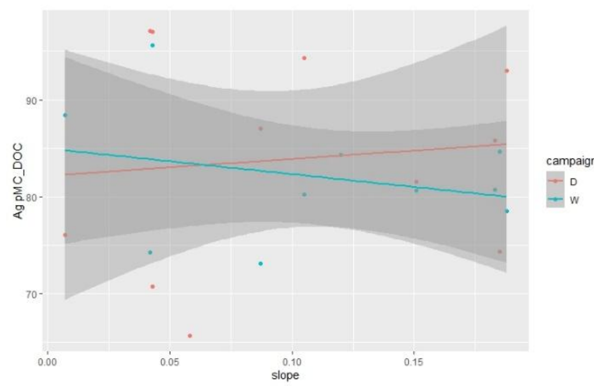
I understand that meaningful relationships identified in a GAM are not always apparent in a univariate plot, but it is hard to relate the plot above to the argument that increasing DOC concentrations are associated with decreasing DOC age during the dry season. The argument that older DOC reflects deeper flow paths is also a bit at odds with the age distribution of carbon in core R6 (the one that was considered not to be representative).

Lines 287-289- This is another new result and looks to be from a linear regression of slope vs. DOC age for wet season rainforest sites (for full transparency, I ran this linear regression and got the same R2 as reported here). The R2 values is at odds with the p value, but fortunately, the former is more meaningful than the latter, given N = 6.

Lines 290-294- This is another new result. Given the indication of no relationship between slope and DOC age from Table S2, I generated more plots- with agricultural sites pooled and not pooled for the 2 seasons (this time, confidence intervals were generated using a linear model). Again, I recognize that relationships in partial dependency plots are not necessarily apparent in univariate plots, and that land*slope was used in the GAM. However, this sentence is talking specifically about slope, as is the case for results reported on line 289.

In both cases, these relationships are basically flat and do not support the statement made here that there may be a negative relationship between slope and DOC age.





Lined 306-308- This sentence about aged C being highly biolabile surprised me. In part, it was unexpected because of conventional wisdom that more labile molecules are lost first because they are easier to mineralize, but also because this view is the basis for your third hypothesis (DIC is younger than DOC due to external inputs of young soil CO2 and to younger, more biolabile organic matter being preferentially mineralized). Similarly, leaf litter leachate is described as young and biolabile (lines 331-332).

Line 338- Yes, relatively old DOC was exported during both wet and dry conditions in agricultural streams. But the wording is potentially confusing in the context of Fig. 3a and line 344 that suggest that flow conditions do affect DOC age. Can this be clarified to minimize possible confusion?

Harrell, F.E. 2015. Regression modeling strategies. Springer Nature Publishing.