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Interactive comment on "Lability of natural organic matter in freshwater: a simple method for detection using hydrogen peroxide as an indicator" *by* Isabela Carreira Constantino et al.

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The central focus of this manuscript is to describe a simple assay that utilizes the decay rate of hydrogen peroxide when exposed to natural organic matter (NOM) as a metric to quantify the amount of "labile" material present. This study first measures hydrogen peroxide decay rates in the presence of three laboratory reference materials (lignin, fluvic acid, and pyruvate) at multiple concentrations. Then, using the observation that pyruvate addition enhances hydrogen peroxide decay rate, thus lowering the half-life, the authors generate a model to predict the absolute concentration of "labile" NOM present in a sample, assuming that all "labile" material behaves identically to pyruvate.

All remaining NOM is considered "recalcitrant." This assay is then applied natural samples that have been enriched with the three laboratory reference materials, as well as time-series samples collected from a nearby river.

I have a number of issues with this study, beginning with the overall experimental design and the separation of NOM into "labile" and "recalcitrant" pools. As I understand it, the logic of this experimentation is as follows: (i) choose three reference materials to see if HOOH decays more quickly in their presence, (ii) observe this to be true in the case of pyruvate, (iii) quantify HOOH decay as a function of pyruvate concentration, (iv) assume that pyruvate behaves identically to "labile" NOM, and (v) apply this quantitative relationship to natural samples. I find this logic to be somewhat flawed or, at least, justification is incomplete. For example, why should "labile" NOM in the environment, which presumably contains a complex mixture of compounds and function groups, promote HOOH decay with the exact same kinetic rate constant as pyruvate? Does this imply that only "pyruvate-like" compounds (i.e. those with a ketone and/or carboxylic acid function group) are "labile"? In contrast, it has been shown that dissolved lignin can actually decay quite quickly when exposed to uv light (e.g. Spencer et al. 2009 *JGR*). Additionally, it has been shown that highly condensed aromatic and aliphatic organic substrate is rapidly consumed by heterotrophic microbial communities (e.g. Petsch et al. 2001 *Science*; Hemingway et al. 2018 *Science*). However, according to the experimental design of this study, NOM in both of these cases would be considered "recalcitrant."

This makes me wonder what exactly is meant by "labile" and "recalcitrant." Do these terms refer to the bioavailability of NOM and, if so, why were no incubation experiments done to validate that material promoting rapid HOOH decay is actually consumed quickly by heterotrophic communities? (this is certainly true for pyruvate, but what about natural samples?) Or do these terms refer to lability *with respect to reactive oxygen species* and, if so, how would this translate to bioavailability and persistence in the environment?

Additionally, I find the kinetics of these experiments to be poorly described and poorly justified. Most importantly, I am left wondering why the kinetic order of HOOH decay depends on the chemical composition of OM added (i.e. described as zero-order for fluvic acid and lignin but first-order for pyruvate). For example, for the pyruvate case, is HOOH decay first-order with respect to itself, with respect to pyruvate concentration, or both? As this manuscript is written, I *think* the authors treat this as first-order with respect to itself, but this was not tested. Why was an experiment not done in which the NOM concentration was held constant and initial HOOH concentration was varied? This would easily show the reaction order with respect to HOOH concentration (see, for example, Follett et al. 2014 *PNAS* or Hemingway et al. 2017 *Biogeosciences* for mathematical treatment of these results).

If I am interpreting this correctly, then why would HOOH decay be first-order with respect to itself when pyruvate is added but zero-order with respect to itself when lignin or fluvic acid is added? How could this be translated to a natural sample that contains a complex mixture of compounds? Reaction order would need to be known *a priori*. Rather, it seems to me like a more reasonable kinetic model would be one that is zero-order with respect to HOOH concentration and first-order with respect to oxidizable functional groups present in NOM (although the abovementioned test would need to be performed to validate this). If this is true, then HOOH decay could be described as something like:

$$\frac{d[\mathrm{HOOH}]}{dt} = -k_0 - k_1[\mathrm{NOM}] \tag{1}$$

where k_0 is the "intrinsic" zero-order decay rate without NOM present (termed "control" throughout this manuscript) and k_1 describes the additional HOOH decay promoted by the presences of NOM and is dependent on NOM chemical composition. This would result in a HOOH half-life that scales inversely with NOM concentration (i.e. $t_{1/2} \propto 1/[\mathrm{NOM}]$). This relationship fits the data reported in Table 1 significantly better

than does the model described in Fig. 2 and Eq. 1-2.

Of course, the model I describe here might not be the best one to describe these data, but as this manuscript is currently written I'm not convinced that it's any worse than the model presented in the text. I recommend the authors provide a sound, theoretical justification for their choice of kinetic model and include a mathematical derivation starting from first principles, in addition to the results of any test experiments needed to verify their choices.

Unfortunately, these issues preclude me from recommending publication of this manuscript without a significant overhaul of the experimental design and data interpretation. I would first recommend that the authors reconsider NOM decay dynamics and move away from the simple idea that some material is "labile" while other material is "recalcitrant." Organic matter decay is now known to be an incredibly complex, dynamic process that depends on heterotroph community composition, interaction with minerals and particles, light, temperature, etc. in addition to chemical composition (see, for example, Schmidt et al. 2011 *Nature* for review). The interpretation taken here – i.e. that decay is solely a function of chemical composition and that compounds can be pooled into labile and recalcitrant fractions – is an outdated one.

This isn't to say that the assay described in this manuscript doesn't have potential – it might. However, hydrogen peroxide decay rates can only speak to the chemical composition of organic matter, which is not the same as lability. I suspect that hydrogen peroxide will decay faster in systems with a higher concentration of carboxylic acids (via formation of peroxyacids) and ketones (via the Baeyer-Villiger oxidation and similar reactions). This seems to be validated by the pyruvate experiments, as pyruvate contains both a carboxylic acid and a ketone. If this is true, then this assay might be useful for describing, in a general sense, the chemical composition of NOM functional groups. However, this would require significantly more validation before being used in environmental samples (e.g. by comparing hydrogen peroxide decay rates with ^{1}H and ^{13}C NMR). I believe that this is outside the scope of this manuscript as it is currently

written.

Although I cannot recommend publication of this manuscript without a very serious overhaul, please do not hesitate to contact me for further discussion regarding this review.

Sincerely,

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