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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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We appreciate the valuable time and critical review done by referee 1 and certainly considered the useful comments and suggestions made to improve the manuscript. Please, find attached our answer for specific questions given in point by point as a supplement. Nevertheless, we would like to clarify the main points featured by the referee 1. The method proposed in this paper is new. We based on our experimental approach in previous observations published by Jardim et al (2010), in which it was suggested that H2O2 could be used to distinguish the difference between organic matter incorporated in waters during flooding periods in Negro River (Amazon Basin), but it was not possible to quantify the amount of LOM. These authors used H2O2 kinetic consumption in two samples (freshwater from Negro River and water fortified with fresh leached soil organic matter). They showed a significant change in the chemical speciation of Hg coordinated by redox conditions in aquatic region studied in the presence of labile organic matter (LOM). In the rainy season, there was a great input of allochthonous natural organic matter (NOM) in aquatic bodies, and this NOM, considered fresh and reactive, would be able to scavenge H2O2 naturally photogenerated in the water column, influencing directly the oxidation conditions in this environment. Thus, this comprises one of the direct effects caused by the presence of LOM. In this work, we aimed at the possibility of quantifying labile and recalcitrant organic matter in freshwater samples. This objective was based on the importance that NOM plays in aquatic environment. It is known that NOM plays a relevant role in photoreactions, forming reactive species, or even scavenging these species. It is also primary source of biota and it is able to complex or adsorb other species as well. So, all these abilities are an intrinsic characteristic of NOM and its different reactivity degrees. Due to the complex composition of NOM, it is not feasible to carry out a characterization in molecular level as routine analysis. However, the information about the amount and the temporal variability of LOM in aquatic system would be very useful to explain many different processes in environmental studies. Here, we denominated LOM as NOM that was few oxidized or degraded and it is still able to react as a scavenger of oxidant species in aquatic systems, and it probably represents fresh organic matter input. On the other hand, recalcitrant organic matter (ROM) is the fraction that had already suffered oxidation, and it is less reactive towards oxidant species, such as H2O2. Our approach is different from classical methods used to distinguish organic matter degraded by microorganism or chemically, such as the ones used in the biochemical oxygen demand (BOD) and chemical oxygen demand (COD) measurements, respectively. These methods reflect an estimation of the amount of oxygen necessary to degrade organic content in an aquatic sample. Other approaches in literature are summarized by Filella (2009), but all of them are considered bioassays, such as the ones used to measure the fraction of NOM, assimilable organic carbon (AOC) based on the measurements of growing biomass, and the biodegradable dissolved organic carbon (BDOC) is considered part of dissolved fraction of organic C able to be assimilated by heterotrophic microflora. There is another approach used by Laird and Scavic (1990), in which they measured labile dissolved organic carbon or matter (LDOC or LDOM) by bioassay, so again the lability has been considered the bioavailable fraction of NOM. In our attempt, we led the lability and recalcitrance concepts through the chemical approach, trying to reach a simpler approach than the protocols currently used to determine labile fraction, that consider it as biodegradable fraction of NOM, hence they always include bioassays. Reagents necessary to carry out our approach are quite simple and easily obtained. The time spent on application is also less than the necessary to use a biotic assay. So, we picked out some organic compounds to be tested as models of ROM and LOM, based on their molecular complexity and also on their natural presence in aquatic environments (pyruvate, lignin, ascorbic acid, hydroquinone and fulvic acid). In the specific comments, we added more information about the other tested compounds and why they were not considered good models, that is the reason why they were not presented in this manuscript (see answer of comment 16). Now, we agree that the term best model, used for us in the manuscript can be sustainable for other compounds tested. The results found for ascorbic acid and hydroquinone, other labile model compounds, can be inserted in the new version of the manuscript. For our matter, we simulated the acting of some model compounds, e.g. pyruvate, lignin and fulvic acid as scavengers of H2O2 in controlled system in the microcosm experiments. It is important to add that the kinetic models employed here to determine the order and consequently half-lives of H2O2 were based on the mathematical strict sense of the classical kinetic laws, and it was not our attempt with this experimental approach to discuss about any specific chemical mechanism behind these reactions, once our focus is to apply the mathematical formalism to general systems, such as natural aquatic samples. We are aware that this would be an interesting topic of discussion,

but for this purpose it would be necessary more experiments to establish the rates related not only to H2O2 loss, but also to the other species in the kinetic reactions. Some data treatment was included, as well as we could include all of them as a supplementary material (see answer to comment 21). Considering our definitions, the lignin and fulvic acid had behaved as recalcitrant compounds, because they did not affect the natural consumption of H2O2. In this case, the H2O2 consumption profile followed the same profile presented by control (ultrapure water), indicating that the kinetic of H2O2 decomposition was not affected by the presence of lignin, as well as of the fulvic acid. A distinct behavior in H2O2 kinetic consumption was observed when pyruvate was added. The kinetic consumption was faster as more pyruvate was spiked and we observed the great difference between these microcosms and the control. This meant that pyruvate plays a scavenger role in H2O2 consumption. Thus, we considered pyruvate a good and suitable LOM model. In the next step, we tried to find a correlation between the amount of pyruvate, here represented by TOC (denominated as LOM concentration, see Figure 2 on the manuscript), with the half-live times of H2O2 obtained in the kinetic experiment. We found an exponential equation provide the best fit for these data, leading us to define an equation that allowed to quantify LOM concentration from the half-live times obtained by H2O2 kinetic consumption. Then, we proposed the H2O2 kinetic consumption can be used to quantify LOM content in freshwater samples. We highlighted that organic model compounds considered as recalcitrant, were not used to define a way to quantify recalcitrant organic matter content. They were used to compared the scavenger effects caused by labile compound in H2O2 loss. Finally, to quantify recalcitrant organic matter concentration in freshwater samples, we suggested this can be calculated by the difference between TOC and LOM. After to define the equations to quantify LOM and ROM amount, we assessed possible matrix effects of environmental sample. For this, we carried out H2O2 kinetic consumption in freshwater collected of Preto river, and also, we did a standard addition of the model compounds, pyruvate (labile model) and fulvic acid (recalcitrant model) in this freshwater sample. Considering that a presence of microorganisms in freshwater samples is ubiquitous, and they comprise a significant sink of H2O2, as related in literature (Pestane and Zika, 1997), we tested also a filtration step using 0.45 μm membrane, to exclude particulate fraction (inorganic and organic) and to minimize the influence of biota in H2O2 loss. For this experiment, profile of disappearance H2O2 was similar to filtered freshwater (only) and that one was spiked with fulvic acid (0.5 and 5.0 mg L-1). These results were expected, as we showed that fulvic acid had not or had few influence as a scavenger specie to H2O2. In the microcosms of freshwater filtered and spiked with pyruvate the kinetic of H2O2 consumption was faster as more pyruvate was added, showing the effect of increment of labile compound, a similar behavior observed in the microcosms conducted with pyruvate solutions in ultrapure water. So, we considered that there was not a matrix effect. Therefore, in this experiment, we did not expect to have a similar behavior only based in kinetic order of H2O2, as it was questioned (see response to comment 26). Since, using a real sample, we did not have a simple system compose by ultrapure water and organic model compounds, there is a mixture of organic compounds and our approach was developed to be sensitive to labile content. Further ahead, we have done this calculation to show that ROM content in this freshwater sample tested was higher than LOM. Beyond this, we applied this approach develop in the freshwater of Preto river during a year, to quantify LOM and ROM concentrations and also to related these amounts with seasonal effects. Finally, we would like to reinforce that we agree on the rewriting of some topics in this manuscript would be meaningful and this is feasible to carry out. Please, find attached our answer to specific comments and some suggestions to make some of these topics clearer in the supplement.

Please also note the supplement to this comment:
https://www.biogeosciences-discuss.net/bg-2018-122/bg-2018-122-AC1-supplement.pdf

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