

Response to Anonymous Referee #2 regarding their review of “Sediment phosphorus speciation and mobility under dynamic redox conditions”, which was published on February 19th 2017

We thank the referee for his/her careful and thorough review of our manuscript. We appreciate their very detailed comments particularly considering interpretation of the sequential extraction and ³¹P NMR results. The references and comments provided will allow us to significantly improve the quality of the final manuscript. We agree with all of the referee’s comments and will take actions to address their concerns in a revised version of the manuscript.

General comments:

“The manuscript is well written, however, their aims of study (Line 79-87) don’t match their major findings. Even though their aims were focusing on organic P cycling, their main findings were not likely related to organic P cycling, but the inorganic P sorption/desorption mechanisms related to redox chemistry. Their implications didn’t include anything related to organic P cycling. Besides, I am not certain if their experimental design (individual algal additions) was reasonable to represent their study site for organic P cycling. Therefore, I recommend that the aims of the study be re-written.”

We agree with the reviewer, although we believe that lines 79-81 (repeated below) are a good reflection of the aims of the project, the list of particular aims on lines 81-87 do not match the most novel findings from this study. We will re-write this section to better reflect the key points from experimental results in the revised version of the manuscript.

“The aim of this study is to elucidate the microbial and geochemical mechanisms of in sediment phosphorus cycling and release associated with commonly occurring short redox fluctuations (days) at the SWI in shallow eutrophic environments.”

Detailed comments/suggestions:

Clarification of the additional step in the extraction protocol (1M NaHCO₃) –

We think that the reviewer has raised an important point, which we agree requires further clarification in the manuscript. The 1M NaHCO₃ extract was mildly brown in color and we will definitely include that observation in the revised version of the manuscript. We agree that our initial assertion that this extraction corresponds uniquely to humic-metal ternary complexes is likely over simplification/over interpretation. Ultimately, of course this is an operationally defined P fraction, but we agree that further discussion of what this fraction represents would be useful,

particularly as it accounts for such a large proportion of TP. We agree that this fraction likely includes some Ca-P and Mg-P as well as some organic-P. We did in fact measure Ca, Mg, Mn and Fe on each extract and we propose to include these results within the supporting information of the manuscript. We also measured SRP and TDP on the extracts but chose not to include the SRP results, as we do not feel confident that the initial SRP/TP ratio in the solid phase would be preserved during the extractions. If the reviewer feels that this information would be useful to readers we would be happy to include it within the main manuscript. We believe that references provided by the reviewer, as well as their insightful comments within their review, will allow us to improve this aspect of the manuscript in a revised version. We will also include the reaction time for each extract within the supplementary material to allow more direct comparison to extracts such as Olsen P and Hedley's.

Even though the authors mentioned Fe/Mn-oxides in their abstract, discussion was made only for Fe-oxides but nothing for Mn-oxides

We agree, this is an oversight. We did include measurements of Mn in the aqueous phase (Figure 3) and for each of the extracts but this data was not presented or discussed in the manuscript. We propose to include the Mn extract data in the supporting information, and extend the discussion to include extracted Ca, Mg, Mn and Fe for the key extraction steps.

Accumulation of polyphosphate or/and pyrophosphate is known to occur as a luxury uptake in algae and microbes (Hupfer, Gloess, & Grossart, 2007) and is often observed in the surface sediments (Giles, CadeMenun, & Hill, 2011; Hupfer et al., 2007; Jorgensen, Inglett, Jensen, Reitzel, & Reddy, 2015; Li et al., 2015), however, the accumulation of poly- or/and pyrophosphate was not observed in their experiment. The authors commented this was because the experimental condition was not set in excess P (Line 373), despite the actual sampling site being consistently high in primary productivity (Line 369). I am not certain if the conclusion of the polyphosphate cycle in their experimental setting was valid.

The reviewer has highlighted the need to clarify the text in regard to this point. The TP concentrations in the sediments at the field site are high (Bowman and Theysmeyer, 2014), and certainly contribute significantly to the high primary productivity observed at the site via internal loading (Chow-Fraser et al., 1998) due in part to variable redox conditions. Consequently, the TP concentrations in the reactor are high at all times throughout the experiment. The statement on line 373 refers only to the fact that the algal matter added during the experiment, as a source of organic carbon to fuel metabolic processes, did not represent a major contribution of phosphorus to the system. This should not be taken to mean that P is highly limiting in the system at all times. As TDP concentration is very high during anoxic conditions and very low during oxic conditions, luxury uptake required for polyphosphate production, which is known to occur under oxic conditions, cannot occur. This is despite very high TP

concentrations, periodically high TDP concentrations and oscillatory redox conditions. We believe that this observation of the competition between luxury microbial uptake and immobilisation within redox sensitive minerals is a valuable contribution to the literature.

(2) Some peaks, especially at chemical shifts assigned as alpha- and beta-glycerophosphates, appearing in the monoester regions often belong to phosphodiester (Doolette, Smernik, & Dougherty, 2009; Jorgensen et al., 2015; Paraskova et al., 2014; Turner, Mahieu, & Condron, 2003). It is important to consider re-calculating these peaks when comparing the ratio of monoesters to diesters, if the authors didn't do it.

We did not apply this correction but will do so in the revised version of the manuscript. We thank the reviewer for bringing this to our attention.

(3) The recovery rate of total P by the NaOH-EDTA was not shown in the manuscript.

We apologise for this omission, the recovery will be included in the revised version of the manuscript.

Line 153: An explanation of the reason why the setting (i.e. temperature at 25 C and the dark setting) was chosen should be noted.

We agree with the reviewer. The average temperature at the SWI at the sampling location averaged 23.8°C in August 2014, we chose 25°C as it is close to field values, at least for summer conditions, and allowed for rapid biogeochemical cycling, which was the focus of this study. Dark conditions were chosen as the conditions were designed to simulate cycling in the top 12cm of sediment, which are rapidly turned over due to bioturbation. Dark conditions prevail below the surface at all times and during the growing season at the sediment-water interface due to the growth of thick algal mats on the water surface. We will include this rationale in the revised version of the manuscript.

Line 192-194: I suggest moving these sentences to introduction or discussion sections.

We agree with the reviewer, this sentence will be moved to the discussion in the revised version of the manuscript.

Line 198: It is not clear which sediment profile was used for the SEDEX (I imagine it was the surface sediment; 0-4 cm). The number of samples used for the SEDEX should be noted. It would be useful to include a table showing a brief method (i.e. each extractant, pH, shaking time, number of wash, etc.) for readers, since the SEDEX method used in their experiment was modified.

We apologise for this lack of clarity. The SEDEX protocol was applied to solid phase samples taken from the reactor during the laboratory experiment. The sediment used in the reactor was sampled from 0-12cm sediment depth in the field (as stated on line 129). A duplicate of each sample was analysed through SEDEX, the average of these two samples is presented. We will include these details and reword this section for improved clarity in the revised version of the manuscript. We will additionally include a table, as suggested by the reviewer, detailing the extraction procedure.

Tables for basic physical/chemical characteristics of (1) sediment sample, such as pH, texture, TP, NaOH-EDTA extractable P, OM, N and other elements (i.e. Ca, Fe, Al, Mn, and (2) surface water sample such as pH and chemical contents would be useful to readers.

Agreed, we will include these tables in the revised manuscript.

Line 209: PCFA should be expressed as Ca-bound P since the fraction includes not only CaCO₃-associated P, but authigenic carbonate fluorapatite and biogenic apatite. Ruttenberg (1992) reported that the first step (MgCl₂) can extract ~25% of biogenic CaCO₃ (i.e. loosely sorbed P onto CaCO₃).

We agree with the reviewer. We propose to revert to the name applied to this extraction by Ruttenberg in the original method.

Line 339: The pH data obtained during the experiment during anoxic and oxic states would be useful for readers.

We agree and propose to include time series pH data within Figure 3.

References:

Bowman, J., Theysmeyer, T., 2014. 2013 RBG Marsh Sediment Quality Assessment (No. Report No. 2014-14). Royal Botanical Gardens, Burlington, Ontario.

Chow-Fraser, P., Loughheed, V., Le Thiec, V., Crosbie, B., Simser, L., Lord, J., 1998. Long-term response of the biotic community to fluctuating water levels and changes in water quality in Cootes Paradise Marsh, a degraded coastal wetland of Lake Ontario. *Wetl. Ecol. Manag.* 6, 19–42. doi:10.1023/A:1008491520668