

## ***Interactive comment on “Forms of organic phosphorus in wetland soils” by A. W. Cheesman et al.***

**A. W. Cheesman et al.**

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The authors are gratified that the anonymous referee finds the paper well written and with sufficient detail to assess the quality of the data and our interpretations critically. We welcome the opportunity to address specific comments raised, with our comments (in bold) listed alongside the referees comments below.

Referee Comment: General comments: It is a well readable paper which presents all relevant data in which the interpretation was based. Phosphorus analysis is a developing field and needs more comparable analysis like this to better assess the natural abundance of different kinds of phosphorus compounds. In the field of  $^{31}\text{P}$  NMR, papers describe the methodology in detail in contrast to many other publications to be found. In this paper, the method section is in detail, well written and understandable

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with even much more information than found in other literature, very good! Specific comments: The statistics used for the site groupings has already been addressed in the previous comment. Further, I have stumbled over the correlations and interpretations leading to the factors reflecting an active vs. an inactive microbial community. It would be helpful to better clarify these interpretations. The statement was that the inorganic polyphosphates correlate positively with microbial biomass for which the conclusion was that the higher the quantity of inorganic polyphosphates the greater the microbial activity, resp. the more active. Or? From reading this text, I was then questioning the role of organic molecules for reflecting microbial activity. In turn, I would expect a higher amount of organic P if a more active microbial community is present. More active microbes = higher amounts of cell wall debris, nucleoside acids etc. Or? Possibly, my assumptions can be addressed by stating the role of inorganic polyphosphates in cell metabolism (indicated in L447-448, why polyphosphates when scarce resource?), their abundance vs. the abundance of the organic load from cell debris and why polyphosphates represent cell activity. In principle, even if correlation is good, does this have an underlying reasoning? And if yes, why activity and not e.g. total microbial abundance?

Authors Response: We believe the referee has misunderstood our position and agree with their caution in linking a particular P composition and microbial 'activity'. Although we found a strong correlation between microbial P and certain P forms (e.g. DNA and inorganic polyphosphate) we are conservative in our interpretation and are careful to put any discussion in the context of microbial biomass rather than microbial activity. We do state "The highly significant correlation between microbial P and long chain polyphosphate may reflect their biological synthesis in response to increased microbial pressure for a critical scarce resource (Harold, 1966; Seufferheld et al., 2008)". However, in the same discussion section (4.3) we also caution against this interpretation given 1) the known interaction between polyphosphate and AEM used in the determination of microbial P and 2) our inability to identify intracellular (live) and extracellular P forms. As set out in section 4.2.3, the role of polyphosphates in eukaryotic

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and prokaryotic cells is not well understood. Long-chain polyphosphates have previously been noted in oligotrophic wetland soils (Ahlgren et al. 2006; Cheesman et al. 2012), but their presence under conditions of extremely low P availability seems counterintuitive. Polyphosphate synthesis could be a mechanism to retain P during periods of static growth or to chelate micronutrients (Harold 1966). However, such mechanisms are currently speculative and would require further investigation. Action: we have changed section 4.3 to read ““The highly significant correlation between microbial P and long chain polyphosphate may reflect biological synthesis of polyphosphate in response to increased microbial demand for a critical and scarce resource (Harold, 1966; Seufferheld et al., 2008)””.

Referee Comment: Technical queries: L 96 Do different treatments (air drying, field fresh) affect results?

Author Response: It has previously been documented that pre-treatment of wetland soils is likely to impact  $^{31}\text{P}$  NMR extraction and spectral analysis (Turner et al., 2007). However, it appears that the impact of pre-treatment on P composition is sample specific, depending on factors such as sample mineralogy. All soil samples within this study were air-dried (considered analogous to a natural drying period) prior to the alkaline extraction step. The difference in sample handling of two European wetland sites resulted in there being no ‘fresh sample’ on which to conduct AEM extractions but did not impact alkaline extraction and NMR analysis. We have addressed this in the revised manuscript by including the text on page 6 line 21 “Although pretreatment is expected to impact P composition in a sample specific manner (Turner et al., 2007) the use of air drying was considered preferable as a means of rapidly stabilizing samples prior to alkaline extraction and  $^{31}\text{P}$  NMR analysis.”

Referee Comment: L 129-130 Does air drying not also possibly change the sample?

Author Response: As noted in the manuscript, any pre-extraction handling is likely to alter P soil composition. We chose to use air-drying as a ‘standard’ and easily reproducible method to stabilize samples.

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Referee Comment: L 233 “difference” without s; Author Response: Corrected

Referee Comment: L 345 “shape” is out of place I think Author Response: Removed

Referee Comment: L 346 Was a correlation done for vegetation and climate? Author Response: As discussed in the results section, vegetation/Cowardin wetland type and basic climatic setting were explored as potential explanatory factors of P composition. However, a multivariate approach including these factors alongside the biogeochemical classification was not attempted. We believe such an attempt with the data presented here runs the risk of over-parameterization and would be better explored using a more targeted (and complete) data set. For example, a large number of acidic high organic matter wetlands systems from a more complete range of global temperatures.

References used in comment

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