Response to **Referee 1 - Interactive comment** on bg-2014-113 “Forms of organic phosphorus in wetland soils” by A. W. Cheesman et al

We are pleased that the referee recognizes the unique and interesting results presented within our paper. Their comments have contributed to an improved manuscript and we describe our point-by-point responses below. Please note, however, that the referee’s comments appear to refer to the original version of the manuscript; which has been revised once in response to comments in an earlier review. This means that some of the referee’s comments have already been corrected and/or adjusted.

Referee comment: My main issue with the paper concerns the way that the initial interpretation of the cluster analysis identifies groups that lead the whole outcomes of the paper. I am not familiar with that type of statistics used to confirm the groupings and, considering the importance of this in determining the rest of the paper, I would like to see a little more presentation of this. At present the abstract states (L19-20) ‘Soil P composition was predicted by two key chemical properties: organic matter and pH’, but then apparently the groups were defined early on by cluster analysis on factors, then lastly the P speciation data are examined in terms of these pre-defined groups. Thus it seems a little as if the groupings lead the process and the relation to the soil P compositions seems in a way ‘retro-fitted’ to these pre-determined groups. However, this is probably just a ‘way of selling the story’ issue in terms of the layout of the paper. If there could be more of a portrayal of (what I’m sure was in reality) an iterative process of site group selection and evaluation of the P compositions that would help. Could you confirm in the methods how this was done?

Author response: As noted by the referee, dealing with a disparate collection of wetland soils poses a challenge regarding how best to compare between and among sites. Instead of simply reporting phosphorus composition within individual sites, we aimed to develop a testable hypothesis based upon the grouping of wetlands into more generalized subsets. This required the reduction of the 28 sites into more general groupings. This process could have been based upon any physical, geographical or biochemical characteristic of interest, but we focused on two groupings: the Cowardin wetland classification (itself a combination of hydrology/vegetation and landscape position) and a combination of two independent biochemical characteristics (organic matter content and pH). Based on previous research on phosphorus in wetlands we presumed that pH and organic matter content would be primary controls on organic phosphorus cycling, although there is no direct evidence of these factors impacting *composition* of phosphorus forms (but see Turner and Blackwell (2013) for an assessment of the influence of pH on soil organic phosphorus composition in terrestrial soils). The phosphorus composition, including the nature and amounts, of organic phosphorus forms present in the site soils, was ‘simplified’ using PCA with the afore mentioned groupings superimposed. We found no significant pattern when superimposing the Cowardin classification (data not presented but discussed) but that there was a significant difference between the four wetland groups delineated by organic matter and pH. This forms the basis of the statistical analysis and subsequent detailed analysis. We believe that the statistical approach is robust, but that the presentation has led to some confusion. To address the referee’s comment, we have adjusted the language in the abstract and methods to help clarify that the conclusions drawn from the study were not pre-empted.

More specific comments and responses

Referee comment: It would be interesting also to learn something of the climate for the different sites as these have a global distribution. Could you give basic climate data in Supplementary Table 1 e.g. average rainfall, altitude and temperature?

Author response: This is an interesting suggestion, we have adjusted the supplementary table 1 to include basic climate data derived from the WolrdClim dataset

Referee comment: Erroneous '?'
Author response: we didn't find this word in the current discussion paper.

Referee comment: "This was considered appropriate given their physical size…' this is unclear.
Author response: adjusted to clarify; see details corrections below.
Referee comment: It is unclear as to how the four surface cores are used in determining the data. **Author response**: this is clarified in the setting out of the sample regime (see below).

Referee comment: Was the sample sieved?
Author response: no "hand processing" involved exhaustive picking using tweezers. Most peats cannot be sieved in the same way as mineral soils.

Referee comment: "If you selected on the basis of these parameters to determine group how could you then conclude that these 2 parameters were predictors of P compositions (ie. Without favouring them through this pre-determination)"
Author response: There appears to be some confusion concerning the relationship between the grouping of wetlands A-D and then the testing of any potential pattern in their phosphorus composition via PCA. The two processes were distinct and the groupings were not influenced by *a priori* knowledge of the phosphorus composition. It merely allowed us to develop a testable hypothesis, that there is a significant difference in phosphorus composition between groups of wetlands. We have adjusted the abstract in the hope to avoid others confusion on this point.

Referee comment: You give the values as means ±1SD. Does this apply to all analyses (even NMR)? L193-218.
Author response: Where given, values are arithmetic mean ± 1 SD. As there was only one NMR spectrum collected at each site based upon an amalgamated sample (see methods) there are no variance terms reported for NMR data. We have adjusted the text to clarify this point (see below).

Referee comment: "Does the Ward's method give you an optimum number of groups? Looking at the data 5 groups (instead of 4) could be conceivable with current group B split into low (9-25% OM) and intermediate (48-69% OM)".
Author response: the Ward's classification system could be used to delineate 5 fundamental wetland groupings; however, we do not believe the parsing of sites into smaller groups would provide additional insight from the analysis of this dataset. Such a study focused on the effects of organic matter within mineral dominated wetlands would of course be interesting, but we believe is better attempted with a more focused data set.

Referee comment: "Could a split have been made in contrast between the parameters of OM and total P (the latter instead of pH)?"

Author response: the wetland sites could have been split on the basis of organic matter and total P content as they do conform to the required 'lack of co-linearity' for the cluster analysis (see Fig 2). However, if you consider the P composition in sites 4,5 and 6 (which are all currently classed as group C wetlands) and which cover a very broad range in total P content there are only limited differences seen in the composition of P forms present. Certainly any differences are likely to be subtle and nothing as striking as the presence/absence of phosphonates in respect to soil pH. We believe the role of total P and P availability on the composition of P forms is better addressed in a more focused study which could control for organic matter and soil pH.

Referee comment: "This might be a naive question but could inorganic orthophosphate be considered biogenic? Is all inorganic ortho-P from rock weathering sources (directly or indirectly through fertiliser P"
Author response: The term 'biogenic' is used to describe P from a biological origin, certainly there is some inorganic orthophosphate held within the cells of biological material (i.e. orthophosphate within cellular vacuoles). Unfortunately it is currently impossible to partition orthophosphate seen in complex environmental samples between that derived from biological pools and that derived from the inorganic matrix. Orthophosphate identified in alkaline extracts of soil is therefore impossible ascribe to either a 'biogenic' or inorganic sources.

Referee comment: 'LOI explain', should be 'explaining'
Author response: done.

Referee comment: "groupings by pH seems to have a less strong basis. Only the 'residual P' (the undefined pool assumed from that determined by difference to be not extracted from the NaOH-EDTA extract compared with total P) really relates to pH differences."
Author response: We disagree with this comment in two regards; first, pH is seen to be fundamental to differentiating the presence/absence of phosphonates within wetland soils, and two the residual p pool is an important consideration. Inversely relating s to the dominance of organic P pools and potentially their importance in the P cycling within wetland soils.

Referee comment: Additional '(' before Cheesman to be removed.
Author response: adjusted in previous version

Referee comment: I think this is 'unable' where presently it says 'able'.
Author response: adjusted

Referee comment: Incorrect spelling of 'magnitude'
Author response: adjusted

Referee comment: Fig. 2. What is the vertical line for in the top part of the figure?
Author response: previously removed from an earlier version.

Referee comment: Fig 9 Error in axis legend
Author response: now figure 6 Adjusted

Specific Changes to manuscript:

Page 8570 line 12 – "Soil P composition was predicted by two key biogeochemical properties: organic matter content and pH."

To read – "Soil P composition was found to be dependent upon two biogeochemical soil properties; organic matter content and pH"

8573 *line 6 – "The 28 wetlands analyzed included a tropical Changuinola peat dome, Panama (Sites 20, 21, and 22) and Houghton Lake treatment wetland, Michigan (sites 4, 5, and 6) in which three separate locations were treated as indi-vidual sites. This was considered appropriate given their physical size (80 and 7km2,respectively) and differences in nutrient status and vegetation types across the wetlands (Cheesman et al., 2012; Kadlec and Mitsch, 2009)"*

To read - "The wetlands analyzed included two wetland complexes, a tropical Changuinola peat dome, Panama and Houghton Lake treatment wetland, Michigan in which three separate locations were treated as distinct wetland sites (sites 20, 21, 22, and sites 4, 5, and 6 respectively). This was considered appropriate given their physical size (80 and 7 km^2, respectively) and differences in nutrient status and vegetation types within each wetland (Cheesman et al., 2012; Kadlec and Mitsch, 2009)"

Pg 8573 line 12 – "Soil sampling consisted of four surface cores (diameter 7.5cm, 10cm deep) collected from independent sites considered representative of the study wetland"

To read- "Soil sampling consisted of four independent surface cores (7.5 cm diameter, 10 cm deep) collected from an area considered representative of the study wetland and analysed for biogeochemical characteristics separately."

Pg 8574 line 1 -… anion exchange membranes (BDH Prolabo® Product number: 551642S, VWR International, UK)

To read – "…anion exchange membranes (AEM: BDH Prolabo® Product number: 551642S, VWR International, UK)"

Pg 8577 line 4 "Presented values represent arithmetic mean of four field replicates ± 1SD with statistical analysis carried out using R (R Development Core Team, 2012)"

To read "Where reported, site specific values represent the arithmetic mean of four field replicates ± one standard deviation. Statistical analysis was conducted using R software (R Development Core Team, 2012)"

Pg 8574 line 11 "..loss on ignition (an…." To read "… loss on ignition (LOI: an estimate.."

Pg 8578 line 14 ".. Site.." to read "..Site 6.."

Pg 8578 line 17 ".. 24.." to read "..23.."

Pg 8582 line 10 " explain" to read "explaining"

Pg 8586 line 22 "(Zilles and Noguera, 2002)" To read " (Zilles at al., 2002)"

Pg 8587 line 14 “.. able..” to read “…unable..”

Pg 8588 line 16 “… magnitude..” to read “..magnitude”

Pg 8596 line 11 additional reference required “ Zilles, J. L., Hung, C. H., and Noguera, D. R.: Presence of *Rhodocyclus* in a full-scale wastewater treatment plant and their participation in enhanced biological phosphorus removal, Water Sci. Technol., 46, 123-128, 2002”

Figure 6. New image required axis legend incorrect

Response to **Referee 2- Interactive comment** on “Forms of organic phosphorus in wetland soils” by A. W. Cheesman et al

Anonymous Referee #2

Received and published: 31 July 2014

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 31P 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 with even much more information than found in other literature, very good!*
Specific comments: The statistics used for the site groupings has already been ad-dressed 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 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 Comment: It has previously been documented that pre-treatment of wetland soils is likely to impact ^{31}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 31P NMR analaysis."

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.

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

Turner, B.L., Newman, S., Cheesman, A.W. & Reddy, K.R. (2007). Sample pretreatment and phosphorus speciation in wetland soils. Soil Sci. Soc. Am. J., 71, 1538-1546.

Ahlgren, J., Reitzel, K., Danielsson, R., Gogoll, A. & Rydin, E. (2006). Biogenic phosphorus in oligotrophic mountain lake sediments: Differences in composition measured with NMR spectroscopy. *Water Res.*, 40, 3705-3712.

Cheesman, A.W., Turner, B.L. & Reddy, K.R. (2012). Soil phosphorus forms along a strong nutrient gradient in a tropical ombrotrophic wetland. *Soil Sci. Soc. Am. J.*, 76, 1496-1506.

Harold, F.M. (1966). Inorganic polyphophates in biology: Structure, metabolism, and function. *Bacteriol. Rev.*, 30, 772-794.